AFLATOXIN ELIMINATION WORKSHOP
Atlanta, Georgia
October 20-22, 1999

Throughout the world, aflatoxin is considered one of the most serious food safety problems. Chronic problems with aflatoxin contamination occur in the southern U.S. cottonseed, corn, peanuts and tree nuts. However, the impact of aflatoxin contamination on the agricultural economy is especially devastating during drought years when aflatoxin affects the Midwestern corn belt. Estimated economic losses in years of major aflatoxin outbreaks have been in the hundreds of millions of dollars.

The Aflatoxin Elimination Workshops have served to bring together the Agricultural Research Service (ARS, USDA), university scientists, and representatives of the cottonseed, corn, peanut, and tree nut industries in a unique cooperative effort to develop aflatoxin control strategies through research and development. The ultimate goal of this effort is to facilitate the commercial implementation of technologies to eliminate the aflatoxin contamination problem in the U.S. marketplace by the turn of the millennium. Most of the research to eliminate aflatoxin is conducted by ARS; however, an important addition to this effort is a competitive award program provided by Congressional appropriations to bring to the research effort the talents of university scientists in cooperation with ARS.

Scientists realized over a decade ago that the aflatoxin problem could not be solved solely by conventional technologies utilized routinely to control the more "typical" plant pathogens. Aflatoxin contamination is a result of fungal infection of host plants by a unique class of microorganisms adapted to subsist saprophytically on organic debris in the field or to infect and produce aflatoxin in living plant tissues. Classical plant disease prevention methods developed to control very fastidious plant pathogens have been generally unsuccessful in excluding aflatoxin producing fungi from their relatively broad ecological niche. The realization of the unique nature of the aflatoxin problem and that novel technologies will be required for its control became a focal point of discussion during strategy development sessions of the first Aflatoxin Elimination Workshop in 1988. Two areas of research and development based on the biology and ecology of *Aspergillus flavus*-group fungi were suggested: (1) novel genetic engineering and/or marker-based breeding methods to enhance general antifungal resistance in crops, and (2) the isolation and formulation of special fungi for use in biocontrol. These biocontrol fungi are strains of *A flavus*-group fungi that do not produce aflatoxin, but have the capability to occupy the same ecological niche in the field and out compete harmful toxin-producing fungi.

The vision of participants in the first Aflatoxin Elimination Workshop has been confirmed by the rapid progress reported in subsequent workshops in developing practical, commercially viable aflatoxin control procedures, based almost entirely on the concepts generally established during the first workshop. Twelve years after the first aflatoxin workshop and on the eve of the new millennium, significant progress has been made toward achieving the goal that was set forth by ARS and Congress to eliminate the aflatoxin contamination problem by the year 2000. This is best exemplified by the efforts of the USDA and Arizona Cotton Research and Protection Council on the development of an area-wide management program based on the application of the atoxigenic A. flavus biocontrol strain in Arizona cotton fields. Data collected to date demonstrate that the atoxigenic

inoculum is being retained in the test plots as well as being dispersed to neighboring plots. All test plots demonstrated significant displacement of the highly toxigenic S strain. Though some initial startup problems were encountered at the atoxigenic strain production facility in 1999, enough inoculum was generated to treat 10,388 acres in Arizona for the 1999 crop year. Cotton modules on both treated and non-treated fields are being tagged for determination of aflatoxin levels in cottonseed. Additionally, saprophytic yeast are being studied as potential biocontrol agents in pistachio and almond orchards while strains of non-toxigenic A. oryzae and A. sojae also show promise as agents for biological control of aflatoxin contamination of peanut.

Natural products continue to be identified that inhibit fungal growth and/or aflatoxin production either directly or indirectly. When feasible, the genes encoding these products have been transferred into plants via plant transformation and regeneration technology. A cultivar of walnut, Tulare, was found to significantly inhibit the growth of *A. flavus* and production of aflatoxin. Determination of the mechanism by which fungal growth and aflatoxin production are inhibited in this walnut cultivar remains to be determined but this breakthrough could possibly lead to identification of a potent antifungal/anti-aflatoxin protein(s) that can be introduced into other crops. Research on both transgenic walnut and peanut plants expressing the *B. thuringiensis cry*1Ac gene has continued to show promise as a means of reducing the levels of insect damage and thus aflatoxin contamination in these crops. Field tests of selected transgenic peanut lines have shown stable expression of cry1Ac over 6 generations and resistance to lesser cornstalk borer. Studies in walnut using the cry1Ac gene under the control of different promoters has shown excellent mortality levels for Codling moth larvae, whose feeding damage leads to aflatoxin contamination of this crop. Efficacy of other antifungal genes such as anionic peroxidase, lipoxygenase, haloperoxidase, and membrane-interacting peptides have also been analyzed in transgenic crops. Inhibition of fungal growth has been observed with immature cottonseed expressing the haloperoxidase or antifungal peptide gene D4E1 using a green fluorescent protein (GFP)-based assay. Additionally, continued progress in the identification and analysis of natural products from plants has resulted in development of a lure that specifically attracts female Codling moths.

The melding of conventional breeding with genetic analysis of heritable traits for resistance to insect and fungal attack has continued to provide insight into possible mechanisms by which plants may defend against these invaders. A number of quantitative trait loci (QTLs) that influence insect and fungal resistance as well as aflatoxin production in select corn inbred lines have been identified and shown to impart some degree of resistance to commercially desirable lines. QTLs influencing corn maysin production and husk tightness have been identified as factors in resistance as have a number of loci from resistant lines such as Tex6 and CI2. Results of these types of studies have led to the conclusion that resistance genes will need to be pyramided into agronomically desirable inbred lines as additive gene action appears to be important for resistance. In addition, a number of African corn lines have been identified that demonstrate resistance levels to fungal invasion that are even higher than the most resistant U.S. lines. Analysis of these African lines (perhaps via the use of proteomics technology) should allow for the identification of proteins and the genes encoding them that impart increased resistance. These genes can then be introduced into agronomically viable U.S. corn lines. Peanut varieties have also been scored for resistance to preharvest aflatoxin contamination and the most promising results were observed with varieties that had

been reported to exhibit drought tolerance. These lines are being crossed with several commercially viable peanut cultivars and breeding lines.

Although biological control, breeding and genetic engineering-based technologies are important themes of the workshops, especially in regard to corn and cotton, it is generally conceded that an integrated approach will most likely be required to control aflatoxin contamination of all susceptible crops. These approaches include areas of research such as crop management and handling practices as well as insect control and fungal-plant relationships in the field. Research has focused on factors such as reducing early hull splitting in pistachio by use of select rootstocks, delaying harvest and improving irrigation techniques. In addition, development of computer-based "expert system" databases such as that for integrated peanut management will facilitate the transfer of data and information on crop management practices to the farmer. Improvements and refinements in preharvest management and insect control practices and postharvest storage and sorting techniques will certainly complement the above mentioned technologies.

Research information summarized in this 1999 workshop proceedings provides the technological foundation for the multiple strategies currently being investigated to eliminate aflatoxin, thus leading to the well grounded optimism that solutions to this serious food safety problem will be available by the beginning of the next century. This innovative aflatoxin control technology under development was made possible only by the ingenious application of research information on the nature of *A. flavus*-group fungi and of the aflatoxin contamination process, knowledge which has been provided over the years by scientists attending the workshop.

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AFLATOXIN ELIMINATION WORKSHOPS

New Orleans, LA	1998
Peoria, IL	1989
St. Louis, MO	1990
Atlanta, GA	1991
Fresno, CA	1992
Little Rock, AR	1993
Atlanta, GA	1995
Fresno, CA	1996
Memphis, TN	1997
St. Louis, MO	1998
Atlanta, GA	1999

COOPERATING COMMODITY GROUPS

Peanuts:	American Peanut Council
Corn:	American Corn Millers Federation National Corn Growers Association Corn Refiners Association
Cottonseed:	National Cottonseed Products Association National Cotton Council
Tree Nut:	Almond Board of California California Pistachio Commission Dry Fruits Association of California Walnut Marketing Board

TABLE OF CONTENTS

AFLATOXIN ELIMINATION WORKSHOP ABSTRACTS OF PRESENTATIONS

Page

CROP RESISTANCE - CONVENTIONAL BREEDING

PANEL DISCUSSION SUMMARY: CROP RESISTANCE - CONVENTIONAL BREEDING 1
Robert Brown, Thomas Gradziel, Baozhu Guo, C. Corly Holbrook, Keith Ingram, Torbert Rocheford, Donald White, Donald Wicklow, and Neil Widstrom
Chaired by Charlie Martinson

PLATFORM PRESENTATIONS:

Elimination of Aflatoxin Contaminated BGYF Kernels Through Conventional Breeding 4
D.T. Wicklow[1] and L.C. Marshall[2]. [1]USDA, ARS, National Center for Agricultural Utilization Research, Peoria, IL and [2] Holden's Seeds (Monsanto), Williamsburg, IA

Endocarp Ventral Vascular Tissue Development Appears To Be the Achilles Heel for Almond Susceptibility To Insect Damage and Aflatoxin Contamination 5
T.M. Gradziel and A. Dandekar, Department of Pomology, University of California, Davis, CA

Evaluation of Mutant B73 and A632 Inbreds for Aflatoxin Reduction and Agronomic Traits 6
C. A. Martinson, Department of Plant Pathology, Iowa State University, Ames, IA

Inheritance of, Molecular Markers Associated with, and Breeding for, Resistance to Aspergillus Ear Rot and Aflatoxin Production in Corn 7
Donald G. White, Torbert R. Rocheford, Gnanambal Naidoo, Chandra Paul, Rebecca D. Rozzi, Dina E. Severns, and Amy M. Forbes; University of Illinois at Urbana-Champaign, Urbana, IL

Pyramiding Ear Resistance to Insects and Invasion by *Aspergillus* spp. for Control of Preharvest Aflatoxin Contamination in Maize 9
N. W. Widstrom[1], A. Butrón[1], B. Z. Guo[1], D. M. Wilson[2], M. E. Snook[3], T. E. Cleveland[4], and R. E. Lynch[1]. [1]USDA-ARS-IBPMRL, Tifton, GA. [2]Univ. of Georgia, CPES. [3] USDA(retired) RRRC, Athens, GA. [4]USDA-ARS-SRRC, New Orleans, LA

RFLP Markers Associated with Silk Antibiosis to Corn Earworm 10
B. Z. Guo[1], Z. Zhang[2], M. E. Snook[2], N. W. Widstrom[1], P. F. Byrne[3], M. D. McMullen[4], and R. E. Lynch[1]; [1]USDA-ARS, Insect Biology and Population Management Research lab, Tifton, GA; [2]University of Georgia, Coastal Plain Experiment Station, Tifton, GA; [3]Colorado State University, Fort Collins, CO; [4]USDA-ARS, Plant Genetics Research Unit, Columbia, MO

Resistance to Aflatoxin Accumulation in Maize Inbreds Selected for Ear Rot Resistance in West and Central Africa 11
Robert L. Brown[1], Zhi-Yuan Chen[2], Thomas E. Cleveland[1], Abebe Menkir[3], Kitty Cardwell[3], Jennifer Kling[3], and Donald G. White[4]. [1]USDA-ARS-SRRC, New Orleans, LA; [2]Louisiana State University, Baton Rouge, LA; [3]International Institute of Tropical Agriculture, Ibadan, Nigeria; [4]University of Illinois, Urbana, IL.

The Search for an Indirect Selection Tool for Resistance to Preharvest Aflatoxin Contamination in Peanut 13
C. Corley Holbrook[1], Kim K. Franke[2], Keith T. Ingram[2], R. Donald Wauchope[1], Huiqin Xue[3], Michael E. Matheron[4], and David M. Wilson[2]. [1]USDA-ARS, Tifton, GA. [2]Univ. Of Georgia, [3]Shandong Peanut Res. Inst., [4]Univ. Of Arizona

Drought and Temperature Effects on Aflatoxin Resistance of Peanut 15
Keith T. Ingram[1], George F. Pateña[1], and C. Corley Holbrook[2]; [1]The University of Georgia, CAES, Griffin, GA. [2]USDA-ARS, Tifton, GA

POSTER PRESENTATIONS:

Summary of Studies Using the Norsolorinic Acid Producing *Aspergillus parasiticus* Mutant to Study Infection and Visually Screen for Aflatoxin Resistance in Corn 16
David M. Wilson[1], Neil W. Widstrom[2], Jason H. Brock[1], Wellington Mubatanhema[1] and Bonnie D. Evans[1], University of Georgia[1] and USDA[2], Tifton, GA

Evaluation of Corn Hybrids for Resistance to Aflatoxin Accumulation 17
W. Paul Williams, Gary L. Windham, and Paul M. Buckley, USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS.

Molecular Genetic Analysis of Resistance to *Aspergillus flavus* in Maize: QTL for Husk Tightness and Silk Maysin in $F_{2:3}$ Lines 18
Ana Butron[1], Baozhu Guo[1], Maurice E. Snook[2], Neil W. Widstrom[1], and Robert E. Lynch[1]. [1]USDA-ARS, Insect Biology and Population Management Lab, Tifton, GA; [2]University of Georgia, Coastal Experiment Station, Tifton, GA

Molecular Genetic Analysis of Resistance to Insects in Maize: QTL For Antibiotic Compounds in Silks 19
Z. J. Zhang,[1] B. Z. Guo,[2] M. E. Snook,[3] N. W. Widstrom,[2] and R. E. Lynch[2]; [1]Department of Entomology, University of Georgia, Coastal Plain Experiment Station, Tifton, GA; [2]USDA-ARS, Insect Biology and Population Management Research Laboratory, Tifton, GA; [3]USDA-ARS, Richard Russell Research Center, Athens, GA

Evaluation of High Oil Vs. Low Oil Corn Hybrids for Aspergillus Ear Rot and Aflatoxin Production 20
D.E. Severns, R.D. Rozzi, D.G. White, and R.J. Lambert, Department of Crop Sciences, University of Illinois, Urbana 61801

Levels of Resistance to Aspergillus Ear Rot and Aflatoxin Accumulation Within F1 Hybrid Combinations of Eight Maize Inbred Resistance Sources 21
G. Naidoo, A.M. Forbes, T. R. Rocheford & D.G. White; Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61820

Summary of Mapping QTLs Associated with Aspergillus Ear Rot and Aflatoxin Accumulation in Multiple Populations 22
G. Naidoo, K. Campbell, A. M. Forbes, A. Hamblin, B. Kaufmann, C. Paul, D. Severns, T. Rocheford & D. White. Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801

CROP MANAGEMENT AND HANDLING, INSECT CONTROL AND FUNGAL RELATIONSHIPS

PANEL DISCUSSION SUMMARY: CROP MANAGEMENT AND HANDLING, INSECT CONTROL AND FUNGAL RELATIONSHIPS 23
Themis Michailides, Thomas Schatzki, and David Wilson
Chaired by Mark Doster

PLATFORM PRESENTATIONS:

Aflatoxin Control in Figs: Cultural Practices and Ecological Relationships 25
Mark Doster[1], Themis Michailides[1], David Goldhamer[2], James Doyle[3], David Morgan[1], and Daniel Felts[1], [1]Dept. of Plant Pathology; [2]Dept. of Land, Air & Water Resources; [3]Dept. of Pomology, Univ. of California, Davis/Kearney Agricultural Center, Parlier, CA

Aflatoxin Control in Pistachios: Reduction of Early Hull Splitting, Decreasing Navel Orangeworm Infestation, and Identifying Contaminated Nuts 26
Mark Doster[1], Themis Michailides[1], Robert Beede[2], and Walter Bentley[1], [1]University of California/Kearney Agricultural Center, Parlier, CA; [2]University of California Cooperative Extension, Kings County, CA

Post-harvest Selection and Sorting for Aflatoxin in Tree and Ground Nuts 27
Thomas F. Schatzki, Thomas Pearson and W.F. Haddon, USDA-ARS-Western Regional Research Center

The Status of Official AOAC International, AOAC Research Institute Certified, and FGIS Protocol Methods for Aflatoxin Determination as They Relate to Current United States, And European Union Aflatoxin Regulations for Grain, Oilseed, Cottonseed and Tree Nuts 28
David M. Wilson[1] and Mary W. Trucksess[2], University of Georgia, Tifton, GA[1], FDA, Washington, DC[2]

POSTER PRESENTATIONS:

Advances in Insect-Oriented IPM of Mycotoxigenic Fungi of Midwest Corn: FY-1999 29
Patrick F. Dowd[1], Robert J. Bartelt[1], Robert W. Behle[1], Mark A. Berhow[1], Jonathan P. Duvick[2], David F. Kendra[3], L. Mark Lagrimini[4], Michael R. McGuire[1], Gary Molid[5], Donald G. White[6]; [1]Bioactive Agents Research Unit, National Center for Agricultural Utilization Research, USDA, Agricultural Research Service, Peoria, IL, [2]Pioneer Hi-Bred, Int., [3]Novartis Seeds, [4]Department of Horticulture and Crop Science, Ohio State University, Columbus (now at Novartis Seeds), [5]Del Monte Food, [6]Department of Crop Sciences, University of Illinois, Urbana

Aflatoxin Accumulation in Transgenic and Nontransgenic Corn Hybrids Infested with Southwestern Corn Borer 31
W. Paul Williams, Paul M. Buckley, Gary L. Windham, and Frank M. Davis, USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS

Comparison of Preharvest Aflatoxin Accumulation in Bt And non Bt Corn in Florida and Georgia 32
David M. Wilson[1], David L. Wright[2], Pawel Wiatrak[2], Don Herzog[2], David Buntin[3] and Dewey Lee[1]. University of Georgia, Tifton, GA[1], North Florida Research and Education Center, Quincy, FL[2], and University of Georgia, Griffin, GA[3]

Occurrence of Fumonisins and Aflatoxins in the South Georgia Corn Survey from 1996 to 1998 33
Wellington Mubatanhema[1], Zeljko Jurjevic[1], David M. Wilson[1], Neil Widstrom[2], Filmore Meredith[3] and Bonnie Evans[1]. [1] University of Georgia, Tifton GA, [2] USDA/ARS, Tifton GA, [3] USDA/ARS, Athens GA.

Fungal Successions and Mycotoxins of Stored Pearl Millet 34
Zeljko Jurjevic[1], David M. Wilson[1], Jeffrey P. Wilson[2], and Howard Casper[3], University of Georgia, Tifton, GA[1], USDA, Tifton, GA[2] and North Dakota University, Fargo, ND[3].

Profile of Aflatoxin and Fumonisin Accumulation in North Carolina Corn 35
Brian Bush, Marty Carson, Marc Cubeta, Winston Hagler, and Gary Payne; North Carolina State University, Raleigh, NC

POTENTIAL USE OF NATURAL PRODUCTS FOR PREVENTION OF FUNGAL INVASION AND/OR AFLATOXIN BIOSYNTHESIS IN CROPS

PANEL DISCUSSION SUMMARY: POTENTIAL USE OF NATURAL PRODUCTS FOR PREVENTION OF FUNGAL INVASION AND/OR AFLATOXIN BIOSYNTHESIS IN CROPS 36
Robert Norton, Nancy Keller, Charles Woloshuk and Gary Payne
Chaired by Bruce Campbell

PLATFORM PRESENTATIONS:

Tree Nut-aflatoxin Interactions: Natural Products Affecting Insect Pests, Growth of *Aspergillus*, Aflatoxigenesis And Aflatoxin Biotransformation 39
Bruce Campbell, Sung-Eun Lee, Douglas Light, Noreen Mahoney, Glory Merrill, Russell Molyneux, and James Roitman. USDA, ARS, Western Regional Research Center, Albany, CA.

Transmission of Aflatoxin B_1 Inhibition in Mycelia of *A. flavus* and Other Inhibitor Studies 42
R. A. Norton, USDA-ARS, NCAUR, Peoria, IL

Linoleic Acid and Linoleic Acid Derivatives Regulate *Aspergillus* Development and Mycotoxin Production 43
Nancy Keller[1], Ana Calvo[1], and Harold Gardner[2]; [1]Texas A&M University, College Station, TX and [2]USDA/ARS/NCAUR, Peoria, IL

Alpha Amylase from *Aspergillus flavus*: Inhibitors Found in Plant Seeds 44
C. P. Woloshuk and Ahmad Fakhoury, Purdue University, West Lafayette, IN

Genetic Studies on the Regulation of Aflatoxin Accumulation 46
Gary A. Payne, North Carolina State University, Raleigh, NC.

POSTER PRESENTATIONS:

Repression of GUS Reporter Constructs of the Aflatoxin Biosynthetic Pathway Genes by Phenolic Compounds 47
Sui-Sheng T. Hua[1], Wanglei Du[2], Gary A. Payne[2], Melanie Flores-Espiritu[1] and James L. Baker[1]. [1]Western Regional Research Center, ARS, USDA, Albany, CA; [2]North Carolina State University, Raleigh, NC

Isoform Patterns of Chitinase and β-1, 3-glucanase in Maturing Corn Kernels (*Zea mays* L.) Associated with *Aspergillus flavus* Milk Stage Infection 48
C. Ji[1], R. A. Norton[2], D. T. Wicklow[2], and P. F. Dowd[2]; [1]Dept. of Crop Sciences, University of Illinois at Champaign-Urbana, Urbana, [2]USDA, ARS, National Center for Agricultural Utilization Research, Bioactive Agents Research, Peoria, IL

Effects of a Novel Compound on Expression of Mycotoxin Biosynthetic Genes 49
Frances Trail, Haixin Xu, Luis Velasquez, and Ray Hammerschmidt. Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan, USA

Advances Toward Development of a Host Plant Volatile-based Attracticide for Codling Moths, a Key Pest in *Aspergillus* Invasion of Walnuts 50
Douglas Light[1], Katherine Reynolds[1], Dayananda Rajapaska[2], Clive Henrick[2], Ronald Buttery[1], Glory Merrill[1], James Roitman[1] and Bruce Campbell[1]. [1] Western Regional Research Center, USDA-ARS, Albany, CA; [2] Trécé, Inc. Salinas, CA

Biotransformation of AFB_1 in Navel Orangeworm and Codling Moth Larvae 51
Sung-Eun Lee, Douglas M. Light and Bruce C. Campbell; USDA, ARS, Western Regional Research Center, Albany, CA

Naturally Active Compounds as Inhibitors of Aflatoxin Biosynthesis 52
Sung-Eun Lee, Noreen E. Mahoney and Bruce C. Campbell. USDA, ARS, Western Regional Research Center, Albany, CA

Effect of Almond Processing on Viability of *A. flavus* Spores 53
N. E. Mahoney, R. J. Molyneux, P. Bayman; USDA, ARS, Western Regional Research Center, Albany, CA

Approaches to a Navel Orangeworm Bioassay for Assessing Antifeedant Properties of Natural Products 54
James N. Roitman; USDA-ARS-Western Regional Research Center, Albany, CA

Comparison of Aflatoxin Production on Defatted Tree Nuts High Anti-aflatoxigenic Activity in Walnut 55
N. E. Mahoney[1], R. J. Molyneux[1], B. C. Campbell[1], G. H. McGranahan[2], and T. M. Gradziel[2], [1]USDA, ARS, Western Regional Research Center, Albany, CA; [2]Deparment of Pomology, University of California, Davis, CA.

Characterization of the Delta-12 Desaturase Gene of *Aspergillus nidulans* and its Relationship with Spore Development and Sterigmatocystin Production 56
Ana Calvo[1], Harold Gardner[2], Nancy Keller[1]; [1]Texas A&M University, College Station, TX and [2]USDA/ARS/NCAUR, Peoria, IL

CROP RESISTANCE - GENETIC ENGINEERING

PANEL DISCUSSION SUMMARY: CROP RESISTANCE – GENETIC ENGINEERING 57
Paul Hasegawa, Anne-Laure Moyne, Gale McGranahan, Arthur Weissinger, Peggy Ozias-Akins, Caryl Chlan
Chaired by K. Rajasekaran

PLATFORM PRESENTATIONS:

Wheat Pathogenesis-Related (Pr)-4 Protein and Osmotin Control of *Aspergillus* Growth 60
Bressan, R.A., Coca, M., Narasimhan, M., Damsz, B., Veronese, P., Huh, G.-H., Salzman, R., Hasegawa, P.M.; Center for Plant Environmental Stress Physiology, 1165 Horticulture Building, Purdue University, West Lafayette, IN 47907-1165

Purification of Two Small Peptides with Antifungal Activity Against *Aspergillus flavus* 61
Anne-laure Moyne[1], Jana Tonder[1], Thomas E. Cleveland[2] and Sadik Tuzun[1]; [1]Plant Pathology Department, Auburn University, Auburn, AL, [2]USDA, ARS, Southern Regional Research Center, New Orleans, LA

Genetic Engineering and Breeding of Walnuts for Control of Aflatoxin 63
Gale McGranahan[1], Abhaya M. Dandekar[1], Patrick Vail[2], Charles Leslie[1], Sandie Uratsu[1] Steven Tebbets[2] and Matthew Escobar[1]; [1]Department of Pomology, 1045 Wickson Hall, University of California, One Shield Ave, Davis CA 95616, [2]Horticultural Crops Research Laboratory, 2021 South Peach Ave; Fresno CA 93727

Inhibition of Fungal Growth by Putative Transgenic Cotton Plants 64
Kanniah Rajasekaran, Jeffrey W. Cary, Tom J. Jacks, Kurt Stromberg, and Thomas E. Cleveland. USDA-ARS, Southern Regional Research Center, New Orleans, LA

Engineering Cotton for Resistance to *Aspergillus Flavus*: Promoters and Structural Genes 65
Caryl A. Chlan[1], Renee Manuel[1], Robert Bourgeois[1], Jeffrey W. Cary[2], Kanniah Rajasekaran[2], and Thomas E. Cleveland[2]; [1]The University of Louisiana at Lafayette, Department of Biology, Lafayette, LA, [2]USDA-ARS, Southern Regional Research Center, New Orleans, LA

Transformation of Peanut with the Defensive Peptidyl Mim¨ D5C 66
A. Weissinger[1], Y.-S. Liu[1], S. Scanlon[1], J. Murray[1], T.E. Cleveland[2], J. Jaynes[3], Eric Mirkov[4] and Francis Moonan[4]; [1]N. C. State University; [2]USDA-ARS, SRRC, [3]Demegen, Inc., [4]Texas A&M University

Genetic Engineering of Peanut: Progress With Bt, Peroxidase, Peptidyl MIM D4E1, And Lipoxygenase 69
Peggy Ozias-Akins[1], Ravinder Gill[1], Hongyu Yang[1], Robert Lynch[2]. [1]Department of Horticulture and [2]USDA-ARS, University of Georgia Coastal Plain Experiment Station, Tifton, GA

POSTER PRESENTATIONS:

Plant Regeneration from Transgenic Peanut Lines (*Arachis Hypogaea* L.) Transformed with a Tomato Anionic Peroxidase Gene 71
R. Gill, H. Yang, and P. Ozias-Akins; University of Georgia Coastal Plain Experiment Station, Tifton, GA

Transformation of Peanut with a Synthetic Antifungal Peptide Gene 72
H.Y. Yang, R. Gill, and P. Ozias-Akins; University of Georgia Coastal Plain Experiment Station, Tifton, GA

Sequence Analysis of a Corn Chitinase from Tex 6 73
Kenneth G. Moore, Arthur K. Weissinger, Rebecca S. Boston, and Gary A. Payne; North Carolina State University, Raleigh, NC

Maize Transformation for Resistance to Aspergillus Ear Rot and Aflatoxin Production 74
Chuansheng Mei, James Wassom, Donald White, and Jack Widholm, Department of Crop Sciences, University of Illinois, Urbana, Illinois

Characterization of an Alkaline Protease Excreted by *Aspergillus flavus* in Infected Corn Kernels 75
Z. -Y. Chen[1], J. W. Cary[2], R. L. Brown[2], K. E. Damann[1], T. E. Cleveland[2]; [1]Dept. Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA, [2]Southern Regional Research Center, USDA-ARS, New Orleans, LA

Characterization of *cssap92*, a Corn Gene Responsive to *Aspergillus flavus* Infection 76
Richard Wilson[1], Harold Gardner[2] and Nancy Keller[1]; [1]Texas A&M University, College Station, TX and [2]USDA, ARS, NCAUR, Peoria, IL

Use of a Green Fluorescent Protein Expressing *Aspergillus flavus* Strain to Assay for Antifungal Factors in Cottonseed 77
Kanniah Rajasekaran, Kurt D. Stromberg, Jeffrey W. Cary, and Thomas E. Cleveland; USDA, ARS, SRRC, New Orleans, LA

Drought Induced Transcriptional Changes in Peanut (*Arachis Hypogaea* L.) 78
Ashok K. Jain and S. M. Basha; Plant Biotechnology Program, Florida A&M University, Tallahassee, FL

MICROBIAL ECOLOGY

PANEL DISCUSSION SUMMARY: MICROBIAL ECOLOGY 79
Larry Antilla, Peter Cotty, Joe Dorner, Bruce Horn, Sylvia Hua, Jay Mellon, and Merritt Nelson
Chaired by Gary Windham

PLATFORM PRESENTATIONS:

Biocontrol Research to Reduce Aflatoxin in Almonds and Pistachios 81
Sui-Sheng T. Hua, Western Regional Research Center, ARS, USDA, Albany, CA

Long-term Influences of Atoxigenic Strain Applications on *Aspergillus flavus* Communities in Commercial Agriculture 82
Peter J. Cotty, Southern Regional Research Center, Agricultural Research Service, United States Department of Agriculture, New Orleans, Louisiana

Production of Commercially Useful Quantities of Atoxigenic Strain Inoculum 83
Larry Antilla[1] and Peter J. Cotty[2]; [1]Arizona Cotton Research and Protection Council, Tempe, AZ and [2]Southern Regional Research Center, USDA, ARS, New Orleans, LA

Potential of the Koji Molds, *Aspergillus oryzae* and *Aspergillus sojae*, as Competitive Fungi in Biological Control of Aflatoxin Contamination 85
Joe W. Dorner, Richard J. Cole, and Bruce W. Horn. USDA, ARS, National Peanut Research Laboratory, Dawson, GA

Sequence of Substrate Utilization During Corn Kernel Infection and Aflatoxin Contamination 86
Jay E. Mellon, Michael K. Dowd, and Peter J. Cotty. USDA, ARS, Southern Regional Research Center, New Orleans, LA

Sentinel Sites for Monitoring *Aspergillus flavus* Community Structure in Southwestern Desert Agricultural Soils 87
Merritt R. Nelson[1], Thomas V. Orum[1], Donna M. Bigelow[1], and Peter J. Cotty[2]; [1]Department of Plant Pathology, University of Arizona, Tucson, AZ, [2]Southern Regional Research Center, USDA , ARS, New Orleans, LA

Role of Vegetative Compatibility in The Biological Control of Aflatoxigenic Fungi 88
Bruce W. Horn, Ronald L. Greene and Joe W. Dorner; National Peanut Research Laboratory, ARS, USDA, Dawson, Georgia

Localization of *Aspergillus flavus* and Aflatoxin Biosynthesis in Developing Ears of Corn 89
G. L. Windham[1], W. P. Williams[1], R. L. Brown[2], T. E. Cleveland[2], and G. A. Payne[3]. [1]USDA, ARS, Mississippi State, MS; [2]USDA, ARS, New Orleans, LA; and [3]Department of Plant Pathology, North Carolina State University, Raleigh, NC.

POSTER PRESENTATIONS:

An *Aspergillus flavus* NRRL 3357 Mutant Producing Synnemata and Stipitate Sclerotia 90
C.E. McAlpin; USDA, REE, ARS, MWA, National Center for Agricultural Utilization Research, Peoria, IL

***Aspergillus flavus* Genotypic Influence on Aflatoxin And Bright Greenish-Yellow Fluorescence of Corn Kernels** 91
D.T. Wicklow, USDA, ARS, National Center for Agricultural Utilization Research, Peoria, IL

Characterization of the *Aspergillus flavus* Population From a California Tree Nut Orchard 92
Sui-Sheng T. Hua[1], Cesaria E. McAlpin[2], James. L. Baker[1] and Crystal. E. Platis[2]. [1]Western Regional Research Center, ARS, USDA, Albany, CA; [2]National Center for Agricultural Utilization Research, ARS, USDA, Peoria, IL

Control of *Aspergillus flavus* in Contaminated Almonds by Saprophytic Yeasts 93
Sui-Sheng T. Hua, James L. Baker and Melanie Flores-Espiritu. Western Regional Research Center, ARS, USDA, Albany, CA

Natural Communities of *Aspergillus* Section Flavi in the Sonoran Desert 94
Maria L. Boyd and Peter J. Cotty. Southern Regional Research Center, Agricultural Research Service, USDA, New Orleans, LA.

Differences in the Occurrence of *Aspergillus flavus* in Peanut in Malawi Tests of Cultivars and Locations 95
Charles T. Kisyombe[1] and David M. Wilson[2]; [1]Ministry of Agriculture, Chitedze Agricultural Research Station, Lilongwe, Malawi and [2]University of Georgia, Tifton, GA

REGULATION OF AFLATOXIN BIOSYNTHESIS

POSTER PRESENTATIONS:

Characterization of *avfA* Involved in the Conversion of Averufin and *OmtB* in the Conversion of Demethylsterigmatocystin and Dihydrodemethylsterigmatocystin Involved in Aflatoxin Biosynthesis in *Aspergillus parasiticus* and *Aspergillus flavus* 96
Jiujiang Yu[1], Charles P. Woloshuk[2], Deepak Bhatnagar[1], and Thomas E. Cleveland[1]; [1]Southern Regional Research Center, USDA, Agricultural Research Service, New Orleans, Louisiana and [2]Department of Plant Pathology, Purdue University, West Lafayette, Indiana

Sugar Utilization Gene Cluster Marks One End of the Aflatoxin Pathway Gene Cluster in *Aspergillus parasiticus* 97
Jiujiang Yu, Perng-Kuang Chang, Deepak Bhatnagar, and Thomas E. Cleveland; USDA/ARS, Southern Regional Research Center, New Orleans, Louisiana, USA

Regulation of *aflR* and *aflJ* 98
Wanglei Du and Gary Payne, North Carolina State University, Raleigh, NC

Characterization of the afl1 Locus in *Aspergillus flavus* by Amplified Fragment Length Polymorphism (AFLP) Analysis 99
Marilee A. Ramesh and Charles P. Woloshuk. Purdue University, West Lafayette, IN

Stimulation of Aflatoxin Biosynthesis in *Aspergillus parasiticus* by cAMP Analogs 100
Ludmila Roze and John E. Linz, Michigan State University, East Lansing, MI

Understanding the How and Why of Aflatoxin Biosynthesis for Toxin Elimination in Crops 101
Deepak Bhatnagar, Jeffrey W. Cary, Perng-Kuang Chang, Thomas E. Cleveland, Kenneth C. Ehrlich, and Jiujiang Yu, USDA/ARS/SRRC, New Orleans, LA

DETECTION, ANALYSIS, AND EXTRACTION OF AFLATOXINS

POSTER PRESENTATIONS:

Rapid and Sensitive Analytical Methods For Aflatoxins: An Update 104
Don Wauchope[1], Annie Ho[2], Corley Holbrook[1], Brad Haney[3], Chris Hassapis[4], Wellington Mubatanhema[2], David Wilson[2]; [1]USDA-ARS, Tifton, GA, [2]Univ. of GA, Tifton, [3]Blue Diamond Growers, Sacramento, CA, and [4]Saddleback Aerospace Corp.

Identification of the Bright-greenish-yellow-fluorescence (BGY-F) Compound on Cotton Lint Associated with Aflatoxin Contamination in Cottonseed 105
Hampden J. Zeringue, Jr.[1], Betty Y. Shih[1], Karol Maskos[2], and Deborah Grimm[2]. [1]USDA, ARS, SRRC, New Orleans LA, [2]Tulane University, New Orleans, LA

SPECIAL PRESENTATION - Development of Expert Systems for the United States Peanut Industry 106
M.C. Lamb[1], J. I. Davidson, Jr.[2], and D. A. Sternitski[3]; [1]Agricultural Economist, [2]Mechanical Engineer, [3]Agricultural Engineer, USDA-ARS National Peanut Research Laboratory, Dawson, GA

APPENDIX I - Workshop Agenda

APPENDIX II - Author Index

APPENDIX III - List of Participants

CROP RESISTANCE - CONVENTIONAL BREEDING

PANEL DISCUSSION

PANEL DISCUSSION TITLE: CROP RESISTANCE - CONVENTIONAL BREEDING

PANEL MEMBERS: Charlie Martinson (Chair), Robert Brown, Thomas Gradziel, Baozhu Guo, C. Corly Holbrook, Keith Ingram, Torbert Rocheford, Donald White, Donald Wicklow, and Neil Widstrom.

SUMMARY OF PRESENTATIONS: Six of the nine presentations involved research on corn, two on peanuts, and one on almonds. The resistance of crops to preharvest aflatoxin contamination has been manifested several ways.

Genetically inherited inhibition of aflatoxin synthesis has been observed by White, Rocheford, Martinson, Brown, and Widstrom in corn germplasm and is one focal point of breeding efforts. Resistance of the corn kernel to invasion by *Aspergillus flavus* has been the more common type of resistance and has been studied extensively by White, Rocheford, Brown, Widstrom and Wicklow. Wicklow has demonstrated that the resistance is manifested in pericarp integrity. Gradziel, working with almonds, found resistance to *A. flavus* growth in advanced breeding stocks. The resistance of corn ears to insect invasion and inhibition of insect development while feeding on the silks was the focus of research by Widstrom and Guo in their efforts to lower preharvest aflatoxin contamination, based on prior information that ear invading insects provide avenues of entry for *A. flavus* and are also vectors of the fungus. Navel orangeworm (NOW) invasion of the almond hull is required for subsequent *A. flavus* invasion. Reductions in preharvest aflatoxin contamination in peanuts in the field was found by Holbrook to be related to drought tolerance of the peanut cultivar.

Martinson discovered inhibition of aflatoxin synthesis in mutagenized B73 and A632 inbred lines and demonstrated that the resistance was effective for reducing preharvest aflatoxin contamination in mutant inbred lines and hybrids of those lines with B100. Some of the mutant selections have the same yield potential as the elite B73 and A632 lines in hybrid crosses and could be used immediately in elite corn belt hybrids. Wicklow has evidence that the frequency of BGYF fluorescing kernels is a phenotypic pericarp trait directly associated with level of aflatoxin contamination and inbred lines vary for frequency of BGYF kernels. Frequency of BGYF kernels in some inbred lines appeared to influence the trait in hybrids. The premise is that BGYF kernels are internally infected kernels resulting from infection of the fungus through cracks and fissures in the pericarp. White and Rocheford have found resistance to both aflatoxin synthesis and invasion by *A. flavus* in the exotic inbreds they are studying and developing. Resistance in CI2 appears to be additive gene action. Attempts to transfer the resistance found in Tex6 and LB31 to elite B73 and Mo17 inbred lines was successful to a degree. None of the converted lines possesses the level of resistance observed in the exotic inbreds. Several regions in the genome of Tex6 have been identifed for significant percentages of the resistance and could be used with marker assisted selection. Marker assisted selection will be needed to pyramid the desirable genes into agronomically desirable inbred lines. Widstrom has initiated a breeding program to pyramid the diverse genes imparting resistance to aflatoxin synthesis, to attack by *A. flavus*, and to insect feeding and development on silks and ear tissues with those providing klendusity through greater and tighter husk coverage on ears, which impedes insect ingression into the ears. Widstrom genetically mapped the cross GTA1 X GT119 for silk-maysin concentration (inhibition of insect development), aflatoxin redution, and husk tightness. Major QTLs were found for maysin concentration and husk coverage. Guo mapped additional populations for maysin concentration. Brown has been searching for resistance to aflatoxin synthesis in germplasm with fungal ear rot resistance from the International Institute of Tropical Agriculture in Nigeria. Based on kernel screening assays Brown has found IITA entries with high levels of resistance.

Resistance to NOW and subsequent aflatoxin contamination of almonds has been found to reside in failure of the endocarp to seal thereby containing volatile attractants of NOW and restricting development of NOW. Breeding lines and varieties have been identified by Gradziel that are resistant to the breakdown of the shell seal.

Measurable physiological reactions of peanut plants to drought stress have been used by Holbrook to select germplasm indirectly for resistance to preharvest aflatoxin contamination of peanuts. Resistant germplasm has a larger root system. Ingram used a minirhizotron to observe directly the infection of peanut pods in situ under various levels of moisture stress and corroborated the importance of drought stresses for *A. flavus* activity. The tagged strain of *A. flavus* used in the minirhizotrons produced a fluorescent protein and could be monitored with the aid of UV light.

SUMMARY OF PANEL DISCUSSION: In response to a question concerning lignification of the almond hull and resistance to NOW and *A. flavus*, Gradziel acknowledged these benefits, but noted that high lignification promotes lower yield, probably due to the high energy requirements of lignin metabolism.

Interest in the role of maysin in resistance to corn earworm prompted a series of responses by Widstrom and Guo that established: 1) maysin is primarily located in corn silks with some in leaves and none in developing kernels, 2) tight husks confine silks to a compressed area within ear and force insects to eat the silks, 3) the ED50 for maysin concentration is 2%(dry wt), 4) maysin inhibits earworm morphogenesis from first instar to second and third instar stages, the latter being the stage preferring kernel feeding, 5) the first and second instars prefer eating silks.

When asked about the protocol for drought stress measurements, Holbrook responded that they are best taken in the morning when differences are greater.

When asked, White indicated that white populations of corn tended to be more resistant than yellow. Martinson indicated that the resistant B73 and A632 mutants resulted from ethyl methane sulfonate treatments and the current materials are in the M8 generation. Martinson and Wicklow stressed that their tests for aflatoxin and BGYF kernels involved iassessments of intact, noninjured kernels. Wicklow relies on naturally cracked kernels for optimum BGYF, but the problem is kernels containing aflatoxin, but have no BGYF.

The minirhizotron employed by Ingram was described in greater detail in responses to inquiries. The use of the tagged fungus and UV illumination allowed him to follow the microbial interactions directly. A digital camera allowed him to record fungal activities through the 2" diameter observation tubes. Magnifications of 10 to 100X were possible, but for depth of field he used 10X most often.

An extensive discussion of gene pyramiding with corn is summarized: Gene pyramiding is a common result of recurrent selection breeding procedures as minor genes are accumulated in the process; detection of these genes is the problem and can be aided greatly by marker assisted selection procedures (Martinson, Rocheford, and White). Families must be evaluated in diverse environments to observe the stability of the materials (Widstrom). Identification of resistant materials must be easily and quickly achieved to speed up the breeding program; therefore a quick system for measuring aflatoxin in many entries is a must (Widstrom). The materials released must be in a form and described adequately so industry can easily use them or transfer the resistance to other germplasm (Widstrom). When developing inbred lines and breeding populations with desirable aflatoxin traits, care must be taken to avoid loss of variability (potential) for other traits, especially yield (White and Widstrom). Cooperation from industry is needed as the mapping is done

in many cases and they need to incorporate the resistance into their proprietary elite inbred lines (White).

The peanut germplasm being investigated now contains entries that look very good for resistance to preharvest aflatoxin contamination and also agronomic traits; they could satisfy the goal for aflatoxin elimination in 2000 according to Holbrook in response to an inquiry of the status of his program. Before release, peanut varieties must satisfy requirements for yield, resistance to other diseases, shelling characteristics, processing, and numerous quality factors. Resistance to preharvest aflatoxin contamination is only one of many desireable traits needed

PLATFORM PRESENTATIONS

ELIMINATION OF AFLATOXIN CONTAMINATED BGYF KERNELS THROUGH CONVENTIONAL BREEDING

D.T. Wicklow[1] and L.C. Marshall[2], [1]USDA, ARS, National Center for Agricultural Utilization Research, Peoria, IL and [2]Holden's Seeds (Monsanto), Williamsburg, IA

The seed coat or testa, underlying the pericarp, is a natural barrier and structural impediment to *A. flavus* infection of the maize kernel germ. Microscopic tears in the seed coat represent a critical susceptibility factor allowing *A. flavus* infection and aflatoxin contamination of otherwise undamaged grain (Smart et al., Phytopathology 80:1287-1294). Previous cooperation with a corn seed company revealed that the harvested grain from *A. flavus* wound-inoculated ears of elite inbreds showed variation for the frequency of undamaged bright greenish yellow fluorescent (BGYF) kernels when examined under ultra violet light (365 nm). The fluorescent kernels are evidence that *A. flavus* has penetrated seed coat defenses and infected the germ. Numbers of BGYF kernels have been related to levels of aflatoxin contamination of grain sampled following harvest (Dickens & Whittaker, J.Am.Oil Chem.Soc.58:973A-975A). Selecting for reduction in the frequency of BGYF kernels in conventional maize breeding programs may result in lines with reduced susceptibility to seed coat tearing and thus provide protection against *A. flavus* and possibly other kernel molds. Using the frequency of BGYF as a selection tool may be less expensive and more convenient than using actual aflatoxin levels.

A set of 43 commercial corn hybrids and 34 inbreds was grown near Monmouth, IL, and hand-pollinated ears were wound-inoculated with *A. flavus*. Kernels in grids surrounding (but not including) the wound-sites were collected and these kernel samples separated into BGYF and non-BGYF portions of each sample. Generally, most of the aflatoxin in the total grid sample came from the BGYF kernels as expected. Removing the BGYF kernels from the sample substantially reduced the aflatoxin level for many hybrids of different genetic backgrounds. While only 12 of the 43 hybrids tested had aflatoxin less that 20 ng/g when the BGYF kernels were included in the sample, 28 of 43 hybrids had aflatoxin less than 20 ng/g in the portion of the sample without BGYF kernels. The number of BGYF kernels/grid for the different hybrids ranged from 0.015 to 2.03, suggesting that there is genetic variability for susceptibility to invasion of the germ by *A. flavus*. Estimates of the average effect of each inbred when used in hybrids were calculated by an unbalanced ANOVA, and also suggest that some inbreds have the effect of reduced BGYF kernels in their hybrids.

Corrrelation between the number of BGYF kernels observed vs the aflatoxin levels was poor for both hybrids and for the estimated effects of inbreds. While reducing the number of BGYF kernels may be an effective strategy for improving aflatoxin resistance in some genetic backgrounds, it seems that it may not be effective for others. 1999 results will be used to confirm the relative differences in the frequency of BGYF kernels between different hybrids and that these differences can be related to the use of certain inbreds. Then we plan to study the differences in BGYF kernel frequency in the progeny of breeding populations made by crossing lines that are relatively high (poor) for BGYF kernels with lines that are low (good), to see whether good lines can be recovered, and whether these "good" lines tend to result in reduced aflatoxin levels from hybrids made with these lines.

ENDOCARP VENTRAL VASCULAR TISSUE DEVELOPMENT APPEARS TO BE THE ACHILLES HEEL FOR ALMOND SUSCEPTIBILITY TO INSECT DAMAGE AND AFLATOXIN CONTAMINATION

T.M. Gradziel and A. Dandekar, Department of Pomology, University of California, Davis, CA

Progress has been made in each of the strategic approaches to the control of preharvest aflatoxin contamination in almond. Targeted areas in this integrated resistance include the suppression *Aspergillus flavus* (AF) infection and control of navel orangeworm *(Amyelois transitella)* (NOW) kernel damage which is prerequisite for AF infection. Progress in developing resistance to NOW has been achieved through the breeding of high endocarp (shell) seal integrity and is being pursued through the breeding/genetic engineering of hull and kernel antibiosis/nonpreference. Resistance to AF has been shown to be present in the undamaged seed coat of several advanced breeding selections and is being further pursued through the breeding/genetic engineering of AF resistance to in the kernel tissue and through the development of genotypes producing low levels of aflatoxin following normal AF infections.

Previous research has shown that conditions which encourage rapid fruit growth during endocarp lignification appear to result in discontinuities in final shell structure and so vulnerability to NOW in most currently important California almond varieties. Breeding lines and varieties have been identified which are resistant to such shell seal breakdown even in conducive environments. Results from 1999 show this resistance to be genetically controlled and with a moderate to high heritability, depending on genetic background. A preliminary structural analysis of developing almond fruit further suggests that failures in endocarp seal often initiate not at the carpel suture as previously believed, but at points parallel to the ventral vascular bundle. Continued aberrant development of this tissue eventually leads to breakdown of the still lignifying endocarp tissue both internal and external to the ventral vascular bundle. Internal tensions during the dessication and hull split of maturing fruit then result in either the complete or partial fracturing of the mature, highly lignified endocarp. The leakage of almond kernel volatiles, possibly including benzaldehyde, then serve to attract both NOW oviposition either on the adjacent hull or directly to the kernel, and subsequent larval feeding on kernel tissue. The observed site of initial development failure suggest the initial vulnerability to be the point of attachment of the funiculus for the normally abortive second almond ovule.

Progress in the development of a transformation protocol for Nonpareil almond include the findings that either sorbitol or fructose are more effective than sucrose for bud-break in tissue culture while sucrose in combination with sorbitol is more effective in supporting shoot elongation compared to either sugar taken individually. A WPM basal media with low auxin/cytokinin proved superior for callus induction. Candidate transgenes, including antifungal and insecticidal compounds are being tested using either the CaMV35S or UBi3 promoters with the walnut somatic-embryo system developed by Drs. McGranahan and Dandekar.

Recent Publications

Abdallah, A., M.H. Ahumada and T.M. Gradziel. 1998. Oil content and fatty acid composition of almond kernels from different genotypes and from different California production regions. J Amer Soc Hort Sci123:633-437.

EVALUATION OF MUTANT B73 AND A632 INBREDS FOR AFLATOXIN REDUCTION AND AGRONOMIC TRAITS

C. A. Martinson. Department of Plant Pathology, Iowa State University, Ames. IA

A total of 8348 M3 families of mutagenized B73 and A632 inbred lines of maize were previously bioassayed for inhibitors of aflatoxin synthesis in hexane extracts of maize seed. Inhibition of aflatoxin synthesis was a rare phenomenon and only six kernels were found. The assay was a destructive test, but through a process of selfing plants grown from residual seed from the same family ear, the frequency of the trait in subsequent families has been concentrated.

In 1998, M7 families of A632 and B73 were selected for definitive field trials based on agronomic traits and uniformity of response towards inhibition of aflatoxin synthesis. Open pollinated and selfed ears on the plants in the field were inoculated at the soft dough stage with *Aspergillus flavus* by injection with a syringe and a 14 gauge Hamilton No.5 point style needle directed tangentially through the developing kernels and along the cob. Ears were allowed to mature on the plant. After harvesting the ear and drying at 35 C, the kernels contiguous with the sporulating inoculation site were shelled. The kernels containing *A. flavus* were assayed for aflatoxin concentration using a modified Velasco minicolumn procedure that would quantitatively detect concentrations of aflatoxin B1 above 5 ppb in the kernels. Aflatoxin was detectable in kernels from 8% of the B73 M7 ears at an average concentration of 6 ppb; by contrast, aflatoxin was detected in 100% of the non-mutagenized normal B73 ears at an average concentration of 583 ppb. Among the A632 M7 families tested, 58% of the infected ears contained detectable levels of aflatoxin in the infected kernels with an average aflatoxin content of 36 ppb. Among normal A632 ears, 100% of the ears had detectable levels of aflatoxin in the infected kernels with an average concentration of 560 ppb.

Plantings in 1999 included the better inbred lines and also hybrid test crosses of the lines with B100. Inoculations of open pollinated ears were made with *A. flavus* spores. Of the samples run to date of this report, only the non-mutagenized inbred lines or hybrids from them have had detectable levels of aflatoxin (>5 ppb). The best B73 families in terms of stable effective resistance to aflatoxin synthesis derive from two original M3 seed. In A632 the best families are from one M3 seed. When evaluated for agronomic traits, most, but not all, of the hybrid test crosses with mutant B73 and A632 yielded as well as the hybrids made from non-mutant B73 and A632.

The better M8 families from each inbred line were selfed for seed increase and possible release to the industry.

The germplasm also tested for resistance to alflatoxin by cooperators in Texas, Lousiana, and Illinois in 1999. At the time of this report only Louisiana had submitted data, and the hybrids from mutant inbreds had significantly less aflatoxin than hybrids from non-mutant lines.

INHERITANCE OF, MOLECULAR MARKERS ASSOCIATED WITH, AND BREEDING FOR, RESISTANCE TO ASPERGILLUS EAR ROT AND AFLATOXIN PRODUCTION IN CORN

Donald G. White, Torbert R. Rocheford, Gnanambal Naidoo, Chandra Paul, Rebecca D. Rozzi, Dina E. Severns, and Amy M. Forbes; University of Illinois at Urbana-Champaign, Urbana, IL

Research at the University of Illinois is divided into four interrelated components including (1) identifying sources of resistance; (2) determining the inheritance of resistance; (3) molecular marker mapping of genes for resistance; and (4) crossing resistance into commercially used B73 and/or Mo17 type inbreds.

Identification of Resistance. We have screened more than 1300 corn inbred lines in F1 crosses with susceptible inbreds Mo17 and/or B73 for resistance to Aspergillus ear rot and aflatoxin production. All screening has been done using inoculation. Additionally, selected sources of resistance have been studied for the past four years in a diallel. All evidence to date indicates that resistant sources Tex6, CI2, OH516, MI82, and Y7 are our best sources of resistance.

Inheritance of Resistance. We have completed our studies with inheritance of resistance from the inbred Tex6. In 1998 and 1999 we concentrated our efforts on inheritance of resistance from inbred line CI2. In this study we included the susceptible parent B73 (P1), the resistant parent CI2 (P2), F1, F2, F3, BCP1, BCP1-self, BCP2, and BCP2-selfed. In the backcross susceptible self generation we evaluated 273 families and the F3 generation 162 families. In 1998 hot weather occurred after inoculation and aflatoxin values of BCP1-self and F3 families ranged from 70 to 1400. This is a good range of reactions for Illinois. Analysis of generation means from 1998 data indicates that additive gene action is important for resistance.

Mapping Genes for Resistance. We have completed molecular marker mapping of Tex6 x FRB73 backcross susceptible selfed and F3 populations. We also grew the F3 population again this summer and used slightly different inoculation procedures in order to generate more phenotypic data. We have detected molecular markers associated with resistance to aflatoxin production in both populations using single factor analysis. We also are using different statistical programs for data analysis with a concentration of effort toward multiple regression and composite interval analysis for all populations. We've identified one region (bngl 118, bin 5.07) associated with 27% of the variation for aflatoxin resistance in the 1996 backcross susceptible self population. Two regions (phi073 bin 3.05 and umc 1038 bin 10.07) account for 27% of the variation for resistance in the F3 population in 1997. These regions were not highly significant in the other year of the study. The phi073 marker is associated with resistance from FR73. The other two markers represent the most promising regions associated with resistance from Tex6 which could be used for marker assisted selection.

Breeding for Disease Resistance. We've made considerable progress in transferring resistance from LB31 and Tex6 into commercially used inbred lines with various degrees of success in obtaining resistance to aflatoxin production and acceptable yield. With LB31 we've crossed resistance into FR1064, which is a widely used B73 type, and FR2128 which is a later maturing B73 type used in the southern United States. Both FR1064 and FR2128 have yellow kernels. We've crossed Tex6 with several commercially used Mo17 and several B73 types and are currently in early generation testing of those backcrosses. We also are backcrossing resistance into white corn using Tex6 and LB31 with commercially used white inbreds FR808 which is a B73 type and FR810 which is a Mo17 type. Unfortunately we cannot recover the high level of resistance that is often seen in

either Tex6 or LB31. Presumably we are not transferring all genes for resistance into commercially used inbreds. It may be that we need to pyramid genes from different sources of resistance into commercially used germplasm in order to achieve high levels of resistance. We've also initiated crossing with Mp313E and Mp420 which are outstanding sources of resistance. however. are extremely late in our environment. With marker assisted selection we may be able to move resistance from Mp313E into a B73 type. Unfortunately, funding is not great enough to maintain the large populations necessary for selection of both high yield and good levels of resistance.

Conclusions. High levels of resistance have been identified and can be transferred into usable germplasm. In most cases, it appears that resistance is under the control of several genes acting in an additive fashion. In order for resistance to have much value it needs to be in commercially usable germplasm. It is unlikely that commercial breeders will use the sources of resistance that have been identified because the resistance is in inbred lines with agronomically very poor characteristics. If we are able to move resistance into inbred lines with good agronomic characteristics, they will not create serious problems in breeding and are more likely to be useful. We strongly believe that genes from the best sources of resistance need to be pyramided into commercially used germplasm related to Stiff Stalk Synthetic (B73) and to Lancaster (Mo17) types in order to create sources of resistance useful in breeding commercial hybrids with high levels of resistance

Severns, D.E., D.G. White and R.J. Lambert. 1998. "Evaluation of high oil hybrids vs. low oil hybrids for resistance to aspergillus ear rot and aflatoxin." Biological and Cultural Tests. 14:9.

Hamblin, A.M., and D.G. White, 1999. "Inheritance of resistance to aspergillus ear rot and aflatoxin production of corn from Tex6." Phytopathology: Accepted

PYRAMIDING EAR RESISTANCE TO INSECTS AND INVASION BY *ASPERGILLUS* SPP. FOR CONTROL OF PREHARVEST AFLATOXIN CONTAMINATION IN MAIZE

N. W. Widstrom[1], A. Butrón[1], B. Z. Guo[1], D. M. Wilson[2], M. E. Snook[3], T. E. Cleveland[4], and R. E. Lynch[1]; [1]USDA-ARS-IBPMRL, Tifton, GA, [2]Univ. of Georgia, CPES, [3] USDA(retired) RRRC, Athens, GA, [4]USDA-ARS-SRRC, New Orleans, LA

Experiments were initiated to achieve several objectives: 1) to identify maize, *Zea mays* L., genotypes in field experiments that when combined, will maximize opportunities for selection of plants with resistance to both corn earworm, *Helicoverpa zea* (Boddie), injury and aflatoxin contamination, 2) to identify QTLs on chromosomes that influence aflatoxin concentration and estimate the contributions of their effects, 3) to identify QTLs on chromosomes that influence resistance to ear-feeding insects, concentration of silk-maysin, and husk traits, and estimate the contribution of their effects, and 4) to utilize all available information to generate a maize population with chemical and physical resistance to *Aspergillus* spp. and ear-feeding insects to be used for extraction of inbred lines using marker-assisted selection. A two-year field experiment to evaluate seven genotypes inoculated with *A. flavus* and infested with corn earworm revealed that significant variation exists among test genotypes for aflatoxin contamination, corn earworm injury, and maturity. The experiment demonstrated that protection of maize ears against aflatoxin contamination is dependent upon several plant traits, including resistance to fungal infection and ear-feeding insects, and excellent husk coverage and tightness. A highly significant correlation was obtained between corn earworm injury and aflatoxin contamination of maize grain. The relationship between ear injury and aflatoxin contamination consistently appears when ear-feeding insects and *Aspergillus* spp. spores are both abundant in the environment. Genetic mapping of cross GT-A1 x GT119 was completed for silk-maysin concentration and husk tightness. DNA genotyping was performed on 250 F_2 plants using 112 RFLP probes and 6 restriction enzymes. Phenotyping has been completed on 250 $F_{2:3}$ families for silk-maysin and husk tightness. The phenotyping of these $F_{2:3}$ families for aflatoxin reduction is now being completed for the randomized complete block design with three replications. Approximately three-fourths of the plot values for maysin were below the mid-parent value while the F_1 was only 20% of that for the high parent (GT-A1). Husk values were grouped around the mid-parent while fewer than 5% were comparable to the tight husked parent (GT119). Major QTLs for maysin were identified on chromosomes 1S and 8L, and QTLs of largest effect for husk tightness were located on chromosomes 4L and 8S. Other QTLs with lesser effects were identified for both maysin concentration and husk tightness, but are probably not significant enough to be helpful during the selection process. GT-A1 will contribute the alleles for low aflatoxin contamination, while GT119 is contributing alleles for husk tightness. Data from these experiments, with other data on these genotypes generated in other experiments, will be used to conduct marker-assisted selection in a maize population to be generated and used for extraction of inbreds with resistance to *Aspergillus* spp. infection and ear-feeding by insects.

RFLP MARKERS ASSOCIATED WITH SILK ANTIBIOSIS TO CORN EARWORM

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The goal of this research is to control/eliminate preharvest aflatoxin contamination of corn through pyramiding resistance genes to corn ear-feeding insects into lines resistant to *Aspergillus flavus* infection and aflatoxin biosynthesis. Genetic control of insects offers an alternative strategy. Host-plant resistance to ear-feeding insects has been identified and characterized as largely due to high maysin in the silks. Insect injury has been consistently associated with increased contamination by aflatoxin and considered as a major contributor to aflatoxin contamination in the fields. We have mapped two F2 population for insect resistance (silk maysin concentration), GE37 x 565 and SC102 x B31857, and have completed mapping of the (SC102 x B31857)F2. Two major QTL markers, *npi286* and *a1*, have been detected for SC102 x B31857. Marker *npi286* close to *p1* locus explained 25.6% of the phenotypic variance and *a1* on chromosome 3L accounted for 15.7% of the phenotypic variance. Using mapping information from GE37 x 565, we have cloned and characterized a new gene in *sh2-a1* region on chromosome 3L which has single or few copies. We have made progress in marker-assisted selection for insect resistance. Using two makers, *p1* and *a1*, we can select high silk maysin lines from backcross populations.

Over the course of this project, we will identify additional maysin-enhancing genes and QTLs associated with the morphological traits, and transfer them into corn lines with resistance to *Aspergillus flavus* and aflatoxin formation using a backcross selection. The effect of the QTLs will be evaluated at multiple locations and in different genetic backgrounds, including both inbreds and hybrids.

RESISTANCE TO AFLATOXIN ACCUMULATION IN MAIZE INBREDS SELECTED FOR EAR ROT RESISTANCE IN WEST AND CENTRAL AFRICA

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76 maize inbreds adapted to savanna and midecological zones of Central and West Africa were sent to SRRC by the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria, as part of a maize germplasm exchange/collaborative research agreement involving the two above-named institutions. The inbreds were then screened for resistance to aflatoxin accumulation, using the laboratory kernel screening assay (KSA). These inbreds had been selected for mid to high resistance to ear rot (*Aspergillus, Fusarium, Diplodia, Botrydiplodia, and/or Macropomina*) under severe inoculum pressure in their respective zones. Results of screening tests showed that one-fourth of the inbreds accumulated aflatoxins at levels significantly lower than the susceptible control (Delta Pine G-4666) used in the screening experiment, and several inbreds supported levels as low as or lower than promising resistant genotypes, GT-MAS:gk or MI82.

Ten inbreds from the KSA screening experiments, 6 potentially resistant and 4 susceptible, were selected for further investigation. Using an *A. flavus* genetic transformant containing a GUS reporter gene linked to a β-tubulin gene promoter in conjunction with the KSA, fungal growth and aflatoxins were both quantified and then compared in the selected lines. Generally, kernels of inbreds supporting lower levels of aflatoxins supported lower fungal growth and vice versa. Exceptions to this were inbred #1368 which accumulated moderately low levels of aflatoxin B_1, yet high levels of fungal growth, and inbred #15 with high aflatoxin levels and low fungal growth. Further studies of inbred lines 15 and 1368 may provide information leading to the identificaton of traits that directly affect the biosynthesis of aflatoxins. Traits previously identified appear to limit aflatoxin production indirectly through fungal growth inhibition.

When SDS-PAGE was applied to kernel extracts of the selected inbred lines, protein profile variations among African lines, and between African lines and "typical" U.S. lines were demonstrated. A closer examination of the14 kDa trypsin inhibitor (TI) revealed no significant differences in TI levels between resistant and susceptible inbreds. This is in contrast to tested U.S. lines, which express higher constitutive levels of TI in resistant genotypes than in ones susceptible to aflatoxin accumulation. Constitutive levels of the 34 kDa Pro-RIP (the inactive form of the ribosome inactivating protein) protein were also quantified and no differences between resistant and susceptible African inbred lines were observed. Quantitative differences between U.S. susceptible and resistant lines have yet to be documented as well, however, levels of Pro-RIP in African lines generally appear to be lower than in U.S. lines. The protein profile diversity demonstrated among African lines and between them and U.S. lines indicates that the potential exists for identifying biochemical markers for resistance to aflatoxin accumulation different from those present in U.S. resistant lines. This could enhance the host resistance strategy of pyramiding different types of resistance traits into agronomically-viable maize germplasm.

IITA maize germplasm should increase the number of resistant lines that are available to U.S. breeding programs. Also, the IITA breeding program can provide germplasm that combine resistance traits of U.S. and African lines and in genetic backgrounds suitable for testing in different areas of the U.S. This breeding program is aided by the existence of three growing seasons per year in West

Africa. The IITA may also be able to provide the U.S. with testing sites naturally high in fungal inoculum, to enhance the realism of resistance trials.

Present and future research includes greenhouse and field testing of potentially resistant lines identified through the KSA, as well as more lab screening of new lines sent by IITA. The recent acquisition of proteomics equipment is expected to enhance our ability to identify, and characterize proteins associated with resistance. The higher resolution of this approach over one-dimensional gels will result in more proteins being identified, and low-expressed proteins being readily detected. Also, the "cleaner" proteins identified using 2 D gels will enhance our ability to clone candidate genes.

References

R. L. Brown, D. Bhatnagar, T. E. Cleveland, and J. W. Cary. 1998. Recent advances in preharvest prevention of mycotoxin contamination. Pages 351-379 in: Mycotoxins in Agriculture and Food Safety. K. K. Sinha and D. Bhatnagar, eds. Marcel Dekker, Inc. New York.

R. L. Brown, Z.-Y. Chen, T. E. Cleveland, and J. S. Russin. 1999. Advances in the development of host resistance in corn to aflatoxin contamination by *Aspergillus flavus*. Phytopathology 89:113-117.

Z.-Y. Chen, R. L. Brown, A. R. Lax, B. Z. Guo, T. E. Cleveland, and J. S. Russin. 1998. Resistance to *Aspergillus flavus* in corn kernels is associated with a 14 kDa protein. Phytopathology 88:276-281.

THE SEARCH FOR AN INDIRECT SELECTION TOOL FOR RESISTANCE TO PREHARVEST AFLATOXIN CONTAMINATION IN PEANUT

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The ultimate goal of this research project is the development of peanut cultivars that have resistance to preharvest aflatoxin contamination (PAC). Our research has resulted in the development of large scale field screening systems that can be used to examine peanut germplasm to identify genes for resistance. We have used these techniques to examine the peanut core collection. The core collection is a representative subsample of the entire U.S. germplasm collection of peanut. The core accessions 66, 99, 147, 158, 215, 276, 282, 511, and 522 appear to have the highest levels of resistance to PAC. These accessions have shown a 70 to 90% reduction in aflatoxin contamination in comparison to susceptible accessions in multiple environments.

We have also examined other genetic material that might possess resistance to PAC. We examined material that had been reported as having resistance to *Aspergillus* based on in vitro studies, material that had resistance to other fungi, and material with low linoleic fatty acid composition. None of this material exhibited a high level of resistance to PAC in our field conditions. More promising results were observed with material that had been reported to have drought tolerance. This material has been documented to have a larger root system and to show relatively less visible stress when subjected to drought conditions. This material also showed a dramatic reduction in aflatoxin contamination. We also observed a significant correlation between leaf temperature and PAC and between visual stress rating and PAC. Leaf temperature and visual stress ratings are less variable and cheaper to measure than PAC, and we hope to be able to use these as indirect selection tools for use in breeding cultivars with resistance to PAC.

We have taken the sources of resistance to PAC that we have identified and crossed this material with several cultivars and breeding lines. Our ultimate goal is to combine the resistance with acceptable yield and grade. We have begun examining these breeding populations under our field testing system for resistance to PAC and will be continuing this work for the next several years.

We are also conducting cooperative studies with Dr. Joe Dorner to examine the effect of combining the use of genetic resistance with the use of biocompetitive fungi. Aflatoxin contamination in these tests at Tifton in 1998 were very low, and results were inconclusive.

Anderson, W. F., C. C. Holbrook and D. M. Wilson. 1996. Development of greenhouse screening for resistance to *Aspergillus parasiticus* infection and preharvest aflatoxin contamination in peanut. Mycopathologia 135:115-118.

Anderson, W. F., C. C. Holbrook, D. M. Wilson and M. E. Matheron. 1995. Evaluation of preharvest aflatoxin contamination in several potentially resistant peanut genotypes. Peanut Sci. 22:29-32.

Cole, R. J., J. W. Dorner, and C. C. Holbrook. 1995. Advances in mycotoxin elimination and resistance. Pp. 456-474. In: Advances in Peanut Science. H. E. Pattee and H. T. Stalker (Editors). Amer. Peanut Res. and Educ. Soc. Stillwater, OK.

Holbrook, C. C., W. F. Anderson, and R. N. Pittman. 1993. Selection of a core collection from the U.S. germplasm collection of peanut. Crop Sci. 33:859-861.

Holbrook, C. C. and W. F. Anderson. 1995. Evaluation of a core collection to identify resistance to late leafspot in peanut. Crop Sci. 35:1700-1702.

Holbrook, C. C., D. M. Wilson, M. E. Matheron, J. E. Hunter, D. A. Knauft, and D. W. Gorbet. 1999. The possible role of fatty acid composition on preharvest aflatoxin contamination of peanut. Plant Dis. Accepted 10/5/99.

Holbrook, C. C., M. E. Matheron, D. M. Wilson, W. F. Anderson, M. E. Will and A. J. Norden. 1994. Development of a large-scale screening system for resistance to preharvest aflatoxin contamination. Peanut Sci. 21:20-22.

Holbrook, C. C., D. M. Wilson and M. E. Matheron. 1997. Results from screening the peanut core collection for resistance to preharvest aflatoxin contamination. Proc. Aflatoxin Elimination Workshop.

Holbrook, C. C., D. M. Wilson, K. S. Ruckers, C. K. Kvien and J. E. Hook. 1994. Possible role of drought tolerance in reducing aflatoxin contamination of peanut. Agron. Abst. p 223.

Holbrook, C. C., D. M. Wilson, M. E. Matheron, and W. F. Anderson. 1997. *Aspergillus* colonization and aflatoxin contamination in peanut genotypes with resistance to other fungal pathogens. Plant Disease. 81:1429-1431.

Holbrook, C. C. 1997. The U.S. germplasm collection of *Arachis hypogaea*: How much diversity do we have? Proc Amer. Peanut Res. and Educ. Soc. 29:64.

Rucker, K. S., C. K. Kvien, C. C. Holbrook, J. E. Hook and D. W. Wilson. 1995. Identification of peanut genotypes with improved drought avoidance traits. Peanut Sci. 22:14-18.

Will, M. E., C. C. Holbrook and D. M. Wilson. 1994. Evaluation of field inoculation techniques for screening peanut genotypes for reaction to preharvest A. flavus group infection and aflatoxin contamination. Peanut Sci. 21:122-125.

DROUGHT AND TEMPERATURE EFFECTS ON AFLATOXIN RESISTANCE OF PEANUT

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Aflatoxin contamination of rainfed peanut is highly variable. Drought intensity, *Aspergillus flavus* populations, and peanut pod development all change dynamically to influence preharvest aflatoxin contamination. With the ultimate goals of developing aflatoxin resistant germplasm and identifying agronomic practices that mitigate aflatoxin contamination, research was conducted with the following objectives: 1) To assess effects of rain exclusion shelters on soil temperature and soil moisture, and whether environmental variability affects variability in aflatoxin contamination; 2) To observe *A. flavus* infection of peanut pods under different intensities of drought; and 3) To develop a quantitative model of the relationships among pod developmental stage, drought intensity, and plant traits.

In aflatoxin screening plots covered by rain exclusion shelters, we monitored the spatial and temporal distribution of soil temperature and moisture distribution at two depths (5 and 25 cm) and 12 locations in 1997 and 1998. We found that the environment beneath shelters was relatively uniform. Two sources of nonuniformity may be avoided by increasing borders, namely along edges of shelters to avoid effects of lateral water movement into the deeper soil layer during periods of high rainfall and in center of west-facing end to avoid high temperature effects caused by afternoon sun exposure. In no case did we find significant correlations between aflatoxin contamination and soil temperature or moisture variation. Thus, we conclude that variation in aflatoxin contamination is mostly a result of genotype differences in aflatoxin resistance.

We used a minirhizotron to observe the progress of *A. flavus* infection of pods for peanut growing in 200-L drums under different levels of drought. We inoculated plants with a green-fluorescent-protein producing strain of *A. flavus* obtained from Jeffrey Cary. Using an ultraviolet light source we observed fluorescence *in situ.* Fluorescence was greater under conditions of low than high water availability for both drought susceptible and resistant varieties. In general, fluorescence faded with time. Even where we observed inoculum directly, fluorescence was not detectable by 3 weeks after inoculation. Our observations do not allow us to determine whether this loss of fluorescence indicates that the *A. flavus* was not actively growing or that it had stopped producing the fluorescent protein.

Using data from these experiments and from the literature we are developing a model to quantify relationships among the various biotic and abiotic components of the peanut-*A. flavus*-aflatoxin system. We plan further development of the model during the next year.

POSTER PRESENTATIONS

SUMMARY OF STUDIES USING THE NORSOLORINIC ACID PRODUCING *ASPERGILLUS PARASITICUS* MUTANT TO STUDY INFECTION AND VISUALLY SCREEN FOR AFLATOXIN RESISTANCE IN CORN

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This series of studies using the red brown color mutant of Aspergillus parasiticus to inoculate corn were initiated in 1994 and were concluded with the 1999 crop year. Aflatoxin synthesis of the norsorlorinic acid (nor) mutant of A. parasiticus is partially blocked. When corn is wound inoculated the fungus infects the corn and produces nor and aflatoxin as well as exhibiting a diagnostic sporulation pattern and color. Many infected kernels can be visually identified because of the reddish color of nor or the distinctive red-brown sporulation pattern. In 1994 there was a highly significant correlation with the red kernel numbers and aflatoxin content. In other years this correlation was not always observed. But the combination of red kernels and fungal sporulation could be used to identify highly susceptible genotypes. The separation of resistant genotypes could not be accomplished using this technique. However, since over 90 percent of the germplasm is susceptible the technique is inexpensive and useful in a screening program to identify potentially resistant germplasm. Studies using the mutant to study infection of corn and aflatoxin production were successful and useful information on infection in Georgia corn was gathered and will be published. Inoculation studies using mixtures of wild type A. flavus and the A. parasiticus mutant were useful in finding out how to effectively inoculate for screening and how the fungi compete in nature. A mixture of 25 percent wild type A. flavus and 75 percent mutant was most often the most effective for establishment of the mutant in inoculated corn ears. The use of the color mutant should save money if applied in genotype screening programs and the information gathered may have direct applications in biological control research.

EVALUATION OF CORN HYBRIDS FOR RESISTANCE TO AFLATOXIN ACCUMULATION

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A potentially effective and highly desirable method of reducing aflatoxin contamination of corn grain is the use of genetically resistant hybrids. This investigation was conducted to determine the level of resistance to aflatoxin accumulation in corn hybrids currently available to farmers in the South. A second objective was to compare the level of resistance to aflatoxin accumulation in commercially available corn hybrids to that of crosses among germplasm lines developed and released as sources of resistance to aflatoxin accumulation by USDA-ARS scientists at Mississippi State, MS and Tifton, GA. In 1998, the hybrids evaluated were selected from those entered in the Mississippi Hybrid Corn Yield Trials. In 1999, several companies contributed hybrids specifically for evaluation for resistance to aflatoxin accumulation. Hybrids were inoculated 7 days after midsilk by injecting an *Aspergillus flavus* spore suspension containing 300,000,000 conidia into the side of the ear. Isolate NRRL 3357 was used as inoculum. Ears were harvested approximately 63 days after midsilk, and the level of aflatoxin was determined using the Vicam Aflatest (Watertown, MA). Aflatoxin levels were extremely high in the Mid South in 1998, and aflatoxin levels in this investigation ranged from 70 to nearly 12000 ppb. Mp313E x Mp494 had the lowest level of aflatoxin. In 1999 when conditions were less conducive to aflatoxin accumulation, aflaoxin levels ranged from 4 to 1062 ppb. Mp313E x Mp715 had the lowest level of aflatoxin. In general crosses with Mp313E, Mp420, and Mp715 as parents exhibited the highest levels of resistance. Although the level of resistance to aflatoxin accumulation of the commercial hybrids was not as high as the resistant check hybrids, there were differences in levels of susceptibility to aflatoxin among the commercial hybrids. The levels of resistance exhibited by the resistant check hybrids indicates that corn hybrids with useful levels of resistance can be produced.

MOLECULAR GENETIC ANALYSIS OF RESISTANCE TO *ASPERGILLUS FLAVUS* IN MAIZE: QTL FOR HUSK TIGHTNESS AND SILK MAYSIN IN $F_{2:3}$ LINES

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The experiment demonstrated that protection of maize ears against aflatoxin contamination is dependent upon several plant traits, including resistance to fungal infection and ear-feeding insects, and excellent husk coverage and tightness. The relationship between ear injury and aflatoxin contamination consistently appears when ear-feeding insects and *Aspergillus flavus* spores are both abundant in the environment. Our long-term goal is to study corn resistance to ear-feeding insects and aflatoxin formation and husk physical traits in order to use marker-assisted selection to pyramid the genes. Genetic mapping was conducted in the cross GT-A1 x GT119. GT-A1 is a inbred line from population GT-MAS:gk has high silk maysin, and resistance to *Aspergillus flavus* invasion and aflatoxin formation, but has loose husks. GT119 has good husk coverage, but has low silk maysin and susceptible to aflatoxin formation. DNA genotyping was performed on 250 F_2 plants using 112 RFLP probes and 6 restriction enzymes. Phenotyping of traits has been completed on 250 $F_{2:3}$ families with randomized complete block design and three replications in the summer of 1999. The phenotyping of these $F_{2:3}$ families for aflatoxin reduction is in process. QTL analysis for silk maysin concentration and husk tightness have been completed. Approximately 3/4 of the plot values for maysin were below the average value of the two parents while the F_1 was only 20% of that for GT-A1. Husk values were around the average value while fewer than 5% were comparable to GT119. Genomic regions on chromosomes 1S, 2L, 4L, and 8L significantly affected silk maysin concentration, and on chromosomes 4L, 6L, 7L, and 8S affected husk tightness. These genomic regions could be used as molecular markers in an assisted-selection program to obtain resistant material to corn earworm attack. Combining QTL for ear insect resistance with those for aflatoxin formation will require additional information on the corn QTL controlling *Aspergillus flavus* infection and aflatoxin production.

MOLECULAR GENETIC ANALYSIS OF RESISTANCE TO INSECTS IN MAIZE: QTL FOR ANTIBIOTIC COMPOUNDS IN SILKS

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Ear-feeding damage by insects has been consistently associated with increased contamination by aflatoxin. Maysin, a C-glycosylflavone in maize silk, has insecticidal activity against corn earworm (*Helicoverpa zea* Boddie) larvae. This study was conducted to identify maize chromosome regions (quantitative trait loci, QTL) associated with maysin synthesis in silks and eventually to apply marker-assisted selection in developing/transferring maysin genes into elite lines with resistance to *Aspergillus flavus*. Using an F_2 populations derived from SC102 (high maysin) and B31857 (low maysin), we examined the genetic mechanisms controlling the synthesis of maysin in silks. Single-factor analysis of variance revealed that *npi286* (near *p1*, Bin 1.03) explained 25.6% of the phenotypic variance and *a1* (Bin 3.09) accounted for 15.7% of the phenotypic variance. Locus *a1* has a recessive gene action for high maysin with the presence of functional *p1* allele. Markers *umc66a* (near *c2*, Bin 4.07) and *umc105a* (Bin 9.02) also detected in this analysis with contribution of 2.8% and 2.3% to the phenotypic variance, respectively. A multiple-locus model, which included *npi286*, *a1*, *csu3* (Bin 1.05), *umc245* (Bin 7.05), *agrr21* (Bin 8.09), *umc105a*, and the epistatic interactions *npi286* x *a1*, *a1* x *agrr21*, *csu3* x *umc245*, and *umc105a* x *umc245*, explained 76.3% of the total phenotypic variance. Tester crosses showed that, at the *a1* locus, SC102 has functional *A1* alleles and B31857 has homozygous recessive *a1* alleles. In cross of GE37 and 65, a major QTL was detected on chromosome 3L and may be a regulator gene in maysin pathway. Using PCR-based strategy, we cloned a gene on chromosome 3L and characterized this gene as a single or low copy gene.

EVALUATION OF HIGH OIL VS. LOW OIL CORN HYBRIDS FOR ASPERGILLUS EAR ROT AND AFLATOXIN PRODUCTION

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Corn grain used for livestock feed has higher value when kernel oil content is greater. This is because the higher oil content of the grain increases the energy concentration in food rations resulting in improved feed efficiency. Most corn hybrids grown in the United States have kernel oil content of approximately 4-5%. Oil percentage can be increased by selection, however, most breeding populations selected for high oil content in grain have not been of value as sources of inbreds for commercial hybrids because of unfavorable agronomic characteristics. Oil content also can be increased using a phenomenon referred to as xenia effects. Xenia is defined as the effect of the pollen parent on the development characteristics of the fruit. With this system seed of a male sterile corn hybrid is planted with 6-8% seed of a fertile high oil (8-12% kernel oil) pollinator. The high oil pollinator will provide the pollen for the male sterile hybrid and produce higher kernel oil in the hybrid kernels. The method has been patented and is referred to as TopCross®. In 1998 we evaluated 15 corn hybrids crossed with high oil inbreds or normal oil content hybrids to determine the effect of kernel oil on susceptibility to Aspergillus ear rot and aflatoxin production following inoculation. Over all hybrids, there was no difference between high oil (4.7% kernel oil) and normal oil (7.7% kernel oil) hybrids with respect to Aspergillus ear rot rating. There was significantly more aflatoxin produced in grain of the high oil hybrids (293.0 ng/g) than normal oil hybrids (183.0 ng/g). The experiment was repeated in 1999 and results will be available in the near future.

One use of high oil corn is in chicken feed. Care will need to be taken to be certain that high oil corn is checked more closely for aflatoxin contamination than normal oil corn given the sensitivity of chickens to aflatoxin and the higher susceptibility of high oil corn to aflatoxin production. It may be that hybrids to be used as high oil hybrids need to be closely evaluated to susceptibility to Aspergillus ear rot and aflatoxin production.

LEVELS OF RESISTANCE TO ASPERGILLUS EAR ROT AND AFLATOXIN ACCUMULATION WITHIN F1 HYBRID COMBINATIONS OF EIGHT MAIZE INBRED RESISTANCE SOURCES

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Six previously identified inbreds associated with reduced ear rot and aflatoxin accumulation, Tex6, CI2, OH516, OH513, LB31 and 75-R001, and two lines, FRB73 and FRMo17, that are relevant to the genetic basis of many Midwest commercial hybrids, were crossed to produce 28 F1 hybrids. The objective of this study was to determine whether or not hybrids created from crosses just among our best resistant sources would outperform any of the crosses of resistant inbreds to FRB73 and FRMo17. These inbreds were selected based on their resistance in hybrid combination with FRB73 and/or FRMo17. The 28 F1 hybrids were evaluated for four years for ear rot infection and aflatoxinB1 content. Ears were inoculated using the pinboard method and a conidial mix of *Aspergillus flavus* isolates, 20-24 days after midsilk. Individual ears were rated for rot, on the percent colonization of the inoculated area. Aflatoxin B1 levels in harvested ears were determined using commercially prepared antibody in an indirect competitive ELISA.

Aflatoxin accumulation was detected in all four years and with a coefficient of variance of 14.68%. Analyses of variance for ear rot and aflatoxin show that differences among hybrids were highly significant for both traits. The general combining ability (GCA) effects for both ear rot and aflatoxin were also significant, as was the interaction between the environment and GCA. The GCA sum of squares suggests that additive effects accounted for more than two thirds of the genetic effect. SCA was mostly non-significant. Tex6, CI2 and OH516 had negative values for both ear rot and aflatoxin. However, only crosses with either Tex6 or OH516 resulted in significantly lower levels of aflatoxin and significant reduction in ear rot infection among the hybrid combinations. GCA values for ear rot and $\log_{10}$ aflatoxin for Tex6 are -7.5173 ($p = 0.0004$) and -0.1549 ($p = 0.0006$) respectively and -6.8988 ($p = 0.0011$) and -0.1543 ($p = 0.0006$) for OH516. These results suggest Tex6, OH516 and CI2 are good sources for marker assisted selection to improve elite lines. Crosses with 75-R001 led to significantly higher amounts of aflatoxin accumulation and significant increases in ear rot infection rates for this germplasm. The data indicates that in some instances the genetic basis of resistance might be non-complementary or similar for some lines as evidenced by poor inhibition of aflatoxin production. Certain lines, however, may have complementary sources of resistance genes, which may offer alternative resistance genes suitable for pyramiding through conventional, and marker assisted selection.

SUMMARY OF MAPPING QTLS ASSOCIATED WITH ASPERGILLUS EAR ROT AND AFLATOXIN ACCUMULATION IN MULTIPLE POPULATIONS

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In order to identify QTLs for aflatoxinB1 and ear rot resistance in Tex6 x FrB73, 173 F3 families and 101 backcross derived Tex6 x B73 BC1S1, families were evaluated for resistance to aflatoxin and ear rot in 1996 and 1997. RFLPs and SSRs were employed to find molecular markers associated with the phenotypic data obtained in 1996 and 1997. Single factor analysis of variance revealed that ear rot resistance and inhibition of aflatoxin were associated with different regions on the maize genome in these two populations. Due to genotype by environment interactions between years the phenotypic data could not be pooled. Markers significantly linked with inhibition of aflatoxin in either one of the years were found on chromosome bins 2.02, 2.09, 3.06, 3.07, 4.08, 9.03 and 10.06 in the Tex6 x B73 F3 and 1.03, 1.06, 5.00-5.01, 5.07, 7.04-7.05 and 10.06 in the BC1S1. For ear rot, markers found on chromosomes 1, 6 and 10 were exclusive to the F3 and those on 3, 4, 7 and 9 were common for ear rot resistance in both the Tex6 x B73 populations. Bins 3.03, 3.04 and 3.05 were important in the F3 and the markers in bin 3.09 in the BC1S1. Two markers from bin 4.03 were significantly associated with ear rot resistance. One of these markers was significant in 1997. Two markers from bin 4.05 were also significantly associated with ear rot resistance. One marker in 4.08 was significant in the F3 families for ear rot resistance.

Further analysis of these datasets using Composite Interval Mapping revealed in 1996 that 27.7% variation was accounted for by 2 major QTLs in F3s of Tex6 x B73.

These markers may be useful in Marker Assisted Breeding to improve resistance to aflatoxin in maize. Markers in Tex6, LB31 and R001 with promising R^2 values will be similarly useful. Chromosomal regions that appear most logical for introgression are around 4.05 and 4.06 in R001 and LB31 respectively. Two regions (5.07 and 10.07) from Tex6 are emerging as likely candidates for introgression.

CROP MANAGEMENT AND HANDLING, INSECT CONTROL AND FUNGAL RELATIONSHIPS

PANEL DISCUSSION

PANEL DISCUSSION TITLE: CROP MANAGEMENT AND HANDLING, INSECT CONTROL AND FUNGAL RELATIONSHIPS

PANEL MEMBERS: Mark Doster (Chair), Themis Michailides, Thomas Schatzki, and David Wilson

SUMMARY OF PRESENTATIONS: Doster presented data on cultural practices and ecological relationships that impacted fungal growth and aflatoxin contamination of California figs. New selections of Calimyrna-type figs demonstated a lower incidence of *Aspergillus* sect. *Flavi* decay than Calimyrna fruit. While burying of drip irrigation lines had no significant effect on fungal decay, it was noted that levels of fungal propagules in the soil and on leaves could be positively correlated with Aspergillus fruit decay. Michailides reported results on aflatoxin control in pistachios with regards to early split formation and effects of removing nuts left after harvest on navel orangeworm (NOW) infestation. Unlike in previous years, a single application of Volck oil in 1999 did not increase early splits compared to non-treated trees. It was also noted that the type of rootstock used affected early split formation. Residual nuts left after harvest were were found to have NOW larvae present in nuts with intact hulls and thus might be a source of NOW infestation and aflatoxin contamination in subsequent harvests. Schatzki reported on post-harvest selection and sorting for aflatoxin in peanuts. A high speed, non-destructive test for aflatoxin in peanut was developed based on analysis of a "dip solution" from peanuts using quadropole mass spectroscopy. Wilson gave and overview of current methods in use by regulatory agencies and industry to test for aflatoxin contamination of commodities. Of most concern was the fact that none of the AOAC methods could reliably measure aflatoxin levels in the 1-5 ppb range which is the current acceptable level for export to the European Union.

SUMMARY OF PANEL DISCUSSION: Widstrom asked whether the negative correlation between dust on fig leaves and fruit decay might be important. Doster responded that the orchards with the highest levels of dust were very different than the other orchards. Michailides said that previous results showed a positive correlation between dust on leaves and fruit decay by *Aspergillus niger*. He added that since more dust correlates with more smut (caused by *A. niger*), this may explain the negative correlation of dust with *A. flavus*. In response to a question about the cause of the difference in decay in first-crop figs and in main-crop figs, Doster said that the differences are probably due to the differences in weather.

Asked about his method, Schatzki said that they used a reversed-phase column. Concerning the extraction of peanuts, he said they dipped the nuts in 60% methanol and 40% water for 2 ½ minutes and that they had tried various times of dipping.

Questioned about whether the EU regulations give methods, Wilson answered that there are some sampling guidelines although no specified method. He recommended using an immunocolumn to clean up and then HPLC, but pointed out that no methods have been verified. In response to the statement by Sadik Tuzun that some peanuts that passed test in Turkey went to Europe and failed test there, Wilson said that EU has no provision for resorting.

Asked about the possibility of using atoxigenic strains in California, Doster said that he was not sure because aflatoxigenic fungi are present in California at very low levels and Michailides said that it will be difficult to see differences when section *Flavi* fungi are only 5-8 CFU/g soil, while in Arizona the numbers are more than 100 CFU/g soil; however, we should try to use atoxigenic strains as biocontrols, otherwise we will not know if we do not try it.

Concerning the feasibility of removing nuts left in pistachio orchards after harvest, Michailides said that this is a standard practice in almond orchards and should be done in pistachio orchards. He also pointed out that with the presence of Botryosphaeria blight disease throughout the pistachio orchards, leading to high number of mummy nuts left on trees, the problem would become worse. Thus, removal of mummies to reduce navel orangeworm should be exercised. He added that several major pistachio growers have been removing mummies by just extrapolating results from almond research and implementing our initial results of our research on navel orangeworm. Doster added that navel orangeworm infestation is worse in almond orchards than in pistachio orchards.

PLATFORM PRESENTATIONS

AFLATOXIN CONTROL IN FIGS: CULTURAL PRACTICES AND ECOLOGICAL RELATIONSHIPS

Mark Doster[1], Themis Michailides[1], David Goldhamer[2], James Doyle[3], David Morgan[1], and Daniel Felts[1], [1]Dept. of Plant Pathology; [2]Dept. of Land, Air & Water Resources; [3]Dept. of Pomology, Univ. of California, Davis/Kearney Agricultural Center, Parlier, CA

Evaluations of first-crop and main-crop Conadria figs collected from two orchards in 1998 were completed. For the years 1996-1998, first-crop figs did not have significantly different levels of decay by *Aspergillus* sect. *Flavi* or aflatoxin contamination than main-crop figs. Nevertheless, first-crop figs might play a role in aflatoxin contamination of main-crop figs, because first-crop figs are more likely to have sporulating colonies on the fig exterior at a time when main-crop figs are becoming susceptible to decay.

The effect of burying drip irrigation lines on fungal decay of figs was investigated. The trees with buried drip irrigation did not have significantly fewer *Aspergillus* sect. *Flavi* propagules in the soil or on the leaves. Furthermore, the treatments did not differ in decay of the fruit by *Aspergillus* sect. *Flavi* . These results might be due to the unusual weather in 1998. Samples of soil, leaves, and fruit have been collected from this experimental plot in 1999 and are being evaluated.

The fruit harvested in 1998 from seventeen new Calimyrna-type fig selections in three commercial orchards had significantly smaller eyes (1.6 mm diameter) than Calimyrna fruit (3.1 mm) but about the same as Conadria fruit (1.8 mm). These fruit from the new selections also had lower incidence of decay by *Aspergillus* sect. *Flavi* (0.03%) than Calimyrna fruit (0.52%). In a research orchard, only one of the new selections had an eye diameter of ripe fruit similar to Calimyrna (3.4 mm), whereas the others had mean eye diameters ranging from 0.7 to 1.9 mm. Fig shoots of ten new selections and Calimyrna were sprayed with a spore suspension of *A. flavus* in a research orchard. All of the new selections had lower incidences of decay caused by *A. flavus* (means ranged from 0.0 to 42.9%) compared to Calimyrna (56.8%). Fruit samples are being analyzed for aflatoxins.

The relationships between various factors (such as densities of propagules in the soil or on leaves) on 27 August and the incidence of fungal decay by aflatoxin-producing fungi at harvest (17 September) were determined for ten commercial Calimyrna orchards in 1998. The levels of propagules in the soil and on leaves were positively correlated with fruit decay caused by *Aspergillus* sect. *Flavi*. Additional samples have been collected in 1999 and are being evaluated.

Publications:

Doster, M.A., T.J. Michailides, D.A. Goldhamer, J. Doyle, and D.P. Morgan. 1999. Fungal Decay of Figs: Effects of First Crop, Irrigation, and New Cultivars on Fruit Decay and Prediction of Smut in Calimyrna Figs. Proceedings California Fig Institute Research, Crop Year 1998. Fresno.

AFLATOXIN CONTROL IN PISTACHIOS: REDUCTION OF EARLY HULL SPLITTING, DECREASING NAVEL ORANGEWORM INFESTATION, AND IDENTIFYING CONTAMINATED NUTS

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In order to determine the effect of delaying harvest on aflatoxin contamination, nut samples were collected on five dates between 14 September and 12 October in a commercial orchard. These samples are being evaluated.

Studies with chemicals that induce pistachio trees to break dormancy early were continued. Unlike previous years, in 1999 a single application of Volck oil sprayed during winter did not increase the formation of early splits compared to the nonsprayed control. These results might be due to the unusually high amount of winter chilling during winter and perhaps lower than normal crop load.

The type of rootstock used affected early split formation. In a trial in Fresno County, trees on *Pistacia atlantica* (4.5%) and PGII rootstock (3.9%) had a substantially higher incidence of early split nuts at harvest than trees on PGI (1.7%) or UCB (1.4%). In another trial in Kern County, trees on PGII rootstock (9.7%) had a higher incidence of early split nuts than trees on *P. atlantica* (6.8%), PGI (6.5%) or UCB (6.5%). In a third trial in Madera County, trees on *P. atlantica* (14.7%) and PGII rootstock (14.5%) had a higher incidence of early split nuts than trees on PGI (11.7%) or UCB (11.3%).

The effect of removing nuts left after harvest on navel orangeworm infestation (a major factor in aflatoxin contamination of pistachio nuts) was investigated in two commercial orchards. In one orchard nuts were removed during pruning (a grower practice to reduce Botryosphaeria blight), while in the other orchard nuts were removed by poling (similar to the practice done in almond orchards). Nut samples have been collected and are being evaluated. Furthermore, samples of nuts left in the orchard after harvest were collected during winter from five commercial orchards. In all five orchards, larvae were present in nuts with intact hulls, whereas prior to harvest larvae are usually only in early split nuts and those nuts with ruptured hulls.

Publications:

Doster, M.A., and Michailides, T.J. 1999. Relationship between shell discoloration of pistachio nuts and incidence of fungal decay and insect infestation. Plant Dis. 83:259-264.

Pearson, T.C., Doster, M.A., and Michailides, T.J. 1999. Automated sorting for pistachio defects by machine vision. Pages 74-75 in California Pistachio Industry Annual Report, Crop Year 1998-99. Fresno.

Doster, M.A., T.J. Michailides, and R.H. Beede. 1999. Effect of rootstock on early splitting. Page 91 in California Pistachio Industry Annual Report, Crop Year 1998-99. Fresno.

Beede, RH., L. Ferguson, M.A. Doster, H.C. Reyes, and J. Padilla. 1999. Effect of rootstock and treatment date on the response of pistachio to dormant applied horticultural mineral oil. Pages 92-93 in California Pistachio Industry Annual Report, Crop Year 1998-99. Fresno.

POST-HARVEST SELECTION AND SORTING FOR AFLATOXIN IN TREE AND GROUND NUTS

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A preliminary aflatoxin distribution in insect damaged Serr walnuts was obtained by measuring the aflatoxin sample distribution of 102 samples of 100 walnuts each. A difficulty was encountered when it was noted that serious HPLC interference occurred from compounds deriving from the kernel skin. Such interfering compounds were not removed by use of immuno-affinity columns preceding the HPLC, but could be countered by using partial HPLC peaks. Only 4 positive samples (4 nuts with aflatoxin 2000-300,000 ppb) were found among the 10200 nuts tested and in light of the difficulty of grinding walnuts, further work on the project was abandoned.

Previously developed peanut sorters are generally incapable of reducing aflatoxin in peanuts by better than 70% (insufficient for current strict market demands), while removing 4% or more of product (too high for commercial reasons). In order to develop a peanut sorter capable of reducing aflatoxin by 90+%, while removing 2-% of product, it is necessary to obtain a training sample of aflatoxin infected peanuts so that we can develop a sorting algorithm. Since one expects but 1 infected nut / approx. 1000 nuts and one would like to have a training set containing 200+ infected nuts, this requires the testing of 200,000+ peanuts non-destructively for aflatoxin. A high-speed, non-destructive test for this purpose was developed, based on dipping the peanuts in extraction fluid without grinding and analyzing the resulting "dip solution" by tandem quadropole MS with autoinjection. By use of this technique over 3400 samples were run during a 10-week period, covering almost 50,000 nuts (many run as sets of 20 / dip). From this sample base 79 nuts were obtained with dip fluid concentrations exceeding approximately 120 ppb. Although these nuts can not presently be analyzed for aflatoxin because such standard analysis would destroy them, we estimate from calibration curves that the aflatoxin levels of the nuts exceed 120 ppb as well. Tests are presently underway to search for features characteristic of aflatoxin among these nuts. This work is funded in part by the Peanut Foundation.

THE STATUS OF OFFICIAL AOAC INTERNATIONAL, AOAC RESEARCH INSTITUTE CERTIFIED, AND FGIS PROTOCOL METHODS FOR AFLATOXIN DETERMINATION AS THEY RELATE TO CURRENT UNITED STATES, AND EUROPEAN UNION AFLATOXIN REGULATIONS FOR GRAIN, OILSEED, COTTONSEED AND TREE NUTS.

David M. Wilson[1] and Mary W. Trucksess[2]; [1]University of Georgia, Tifton, GA, [2]FDA, Washington, DC

AOAC International Official Methods are the methods most desirable for use in commerce because they are reference methods that have been through a collaborative study and the variables that are commonly expected have been documented. Collaboratively studied methods are most often specified by regulating agencies because the operating characteristics have been documented and there is considerable confidence in analytical results from laboratories that have good quality control procedures. Currently the AOAC had three different types of methods. First, the Official Methods are collaboratively studied and refereed before acceptance as Official Methods. Second, the AOAC Research Institute had Certified Methods that have undergone stringent expert review and have been verified by an outside laboratory as meeting the requirements specified in the method. Third, AOAC had the possibility for peer reviewed methods. There are currently several AOAC Official Methods for aflatoxin analysis in various commodities that are used by regulatory agencies and industry. There are also several AOAC Research Institute Certified Methods used by industry and in some cases by regulatory agencies for several commodities. The methods that can be used to test USA export corn for aflatoxin is specified by the FGIS Protocol. Methods accepted by the European Union must meet the European Union criteria and most often this criteria will be documented by carrying out an AOAC collaborative study, having them reviewed and accepted as Official AOAC Methods. Currently the AOAC Official Methods for aflatoxin analysis meet the requirements for use within the United States because all have been tested around the 20 ppb target for total aflatoxins. There are only a few Official AOAC Methods for aflatoxin determination that have been tested at the 1-5 ppb of aflatoxin B_1. Therefore there needs to be a concerted effort to verify methods that can meet the current guidelines for aflatoxin B_1 in the European Union. None of the AOAC Research Institute methods have been certified for use at less than 5 ppb of aflatoxin B_1.

POSTER PRESENTATIONS

ADVANCES IN INSECT-ORIENTED IPM OF MYCOTOXIGENIC FUNGI OF MIDWEST CORN: FY-1999

Patrick F. Dowd[1], Robert J. Bartelt[1], Robert W. Behle[1], Mark A. Berhow[1], Jonathan P. Duvick[2], David F. Kendra[3], L. Mark Lagrimini[4], Michael R. McGuire[1], Gary Molid[5], Donald G. White[6]; [1]Bioactive Agents Research Unit, National Center for Agricultural Utilization Research, USDA, Agricultural Research Service, Peoria, IL, [2]Pioneer Hi-Bred, Int., [3]Novartis Seeds, [4]Department of Horticulture and Crop Science, Ohio State University, Columbus (now at Novartis Seeds), [5]Del Monte Food, [6]Department of Crop Sciences, University of Illinois, Urbana

Resistance

A microbioassay procedure for insects was developed that uses varying size disks cut from thin sheets of cast artificial insect diet. For the smallest disks tested (40 mg rehydrated) enough material is present to feed 10 newly hatched fall armyworm larvae for 3 days. For this disk size, only 40 μgm of material is needed to produce a 1000 ppm wet weight concentration. The diet sheets can also be pressed into 96 well microtiter plates to create uniform disks suitable for microtiter plate reader evaluation of insect assays. In assays using wheat germ agglutinin 625 ppm) and soybean trypsin inhibitor (10,000 ppm) sublethal effects could be detected after 3 days. A protein apparently never tested against insects before caused ca. 70-90% mortality of fall armyworms at 75 ppm after 3 days.

Bt field corn was attacked at about the same level as nonBt field corn at milk stage (including hybrids that expressed the protein throughout the plant) in 1999 by corn earworms (up to 18% in some commercial fields in a hybrid that expressed the protein throughout the plant); few European corn borer larvae were seen at this time. At harvest, the number of sap beetle damaged kernels in a loose husked, exposed tip Bt hybrid that expressed the protein throughout the plant at high levels was as great as caterpillar damage in a Bt hybrid that expressed the protein at low levels in silks and kernels that had better husk coverage. Numbers of caterpillar and sap beetle damaged kernels were ca. 3-fold lower in a Bt hybrid that expressed the protein throughout the plant at high levels compared to the hybrid that expressed the protein at low levels in silks and kernels, in spite of corn earworm presence.

Analysis of mycotoxin levels and insect damage of 1998 samples indicated a high correlation between numbers of insect damaged kernels and fumonisin for most, but not all hybrids. Heavy infestation by corn earworms of Bt hybrids expressing the protein throughout the plant in some areas resulted in only ca. 2-fold lower levels of fumonisin in Bt vs. nonBt corresponding hybrids, although 10X fold reductions were noted in other areas where corn earworm damage was less severe and European corn borers were more common.

Resistance of Tex6 vs. B73 to different insect species was compared in lab and field tests. Flea beetle damage was significantly less to Tex6 vs. B73, but leaf collar feeding of Tex6 by corn rootworm adults was significantly greater than for B73. Little difference in levels of kernel feeding by caterpillars and sap beetle adults was noted for the two inbreds in lab studies. A natural infestation of corn earworms caused only had 15% ear damage in Tex6 compared to 84.1% of B73 ears damage at milk stage. At harvest, the number of damaged kernels per ear, when present, was ca. 3x less for Tex6 (14/ear) than B73 (49/ear). This corn earworm resistance appeared to be due to silk antibiosis in Tex6, since actual caterpillar incidence was about the same as for B73, but caterpillars did not feed very far beyond the husk tips on average by milk stage. Lab studies indicated this resistance was also effective against European corn borers and fall armyworms. High maysin levels appear to be at least partly involved in the silk resistance, although laboratory assays suggest other factors are also involved.

Enhanced tobacco anionic peroxidase which conferred resistance to corn earworms in transgenic tobacco and tomato appeared to be compatible with use of insect nuclear polyhedrosis virus, as indicated by laboratory bioassays. The LD_{50} values, after correction for feeding rates, were either not significantly different, or significantly lower for caterpillars that fed on transgenic vs. wild type tomato and two species of tobacco. Novel antiinsectan compounds were found in transgenic vs. wild type sweetgum expressing tobacco anionic peroxidase (chemical id is pending). The CRADA with Pioneer Hi-Bred, Int. to investigate peroxidase involvement in disease resistance has been extended an additional year.

Monitoring and Biological Control

Simplified baits and traps for sap beetles were found to be as or more precise compared to conventional scouting of Bt and nonBt sweet corn, where sap beetle damage is often equivalent (and the major source of damage on the Bt hybrid). Sap beetle damage was found in up to 68% of the Bt ears in 1999.

The insect pathogen *Beauveria bassiana* was recovered from sap beetles at high rates during fall autoinoculative dispersal, as indicated by isoelectric focusing isozyme fingerprinting analysis of multiple year data.

A predictive microcomputer program for mycotoxin incidence in midwest corn was converted so it would run on Windows 95 and 98. Initial grower distribution has been made to elicit useful feedback.

Publications

Dowd, P.F., Behle, R.W., McGuire, M.R., Nelsen, T.C., Shasha, B.S., Simmons, F.W. and Vega, F.E. 1998. Adherent malathion flour granules as an environmentally selective control for chewing insect pests of dent corn ears: Insect control. J. Econ. Entomol. 91: 1058-1066.

Dowd, P.F., Bennett, G.A. McGuire, M.R., Nelsen, T.C., Shasha, B.S. and Simmons, F.W. 1999. Adherent malathion flour granules as an environmentally selective control for chewing insect pests of dent corn ears: Indirect reduction of mycotoxigenic ear molds. J. Econ. Entomol. 92: 68-75.

Dowd, P.F., Lagrimini, L.M. and Herms, D.A. 1999. Tobacco anionic peroxidase often increases resistance to insects in different dicotyledonous species. Pestic. Sci. 55: 633-634.

Dowd, P.F., Lagrimini, L.M. and Nelsen, T.C. 1999. Relative resistance of transgenic tomato tissues expressing high levels of tobacco anionic peroxidase to different insect species. Nat. Toxins 6: 241-249.

Harry O'Kuru, R.E., Mojtahedi, H., Vaughn, S.F., Dowd, P.F., Santo, G.S., Holser, R.A. and Abbott, T.P. 1999. Milkweed seedmeal: a control for *Meloidogyne chitwoodi* on potatoes. Ind. Crops Prod. 7: 530-558.

AFLATOXIN ACCUMULATION IN TRANSGENIC AND NONTRANSGENIC CORN HYBRIDS INFESTED WITH SOUTHWESTERN CORN BORER

W. Paul Williams, Paul M. Buckley, Gary L. Windham, and Frank M. Davis, USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS

The relationship between insect damage and aflatoxin contamination of corn grain has been examined by several researchers. Because injuries resulting from insect feeding on ears of corn provide potential sites for fungal invasion, resistance to insect damage should be effective in reducing fungal infection and subsequent aflatoxin accumulation. Development of transgenic corn plants expressing insecticidal proteins isolated from the bacterium *Bacillus thuringiensis* (Bt) has provided a new and potentially useful source of resistance to Lepidoptera. The current investigation was undertaken to compare the damage sustained by transgenic and conventional commercial corn hybrids infested with southwestern corn borer, *Diatraea grandiosella,* larvae when plants were in the whorl stage of growth or 14 to 21 days after anthesis and to compare aflatoxin accumulation among hybrids following infestation. The transgenic and nontransgenic versions of seven corn hybrids were included in the investigation. In addition to infestation with insects, the top ears of all plants were sprayed weekly for 5 weeks with an *Aspergillus flavus* spore suspension beginning approximately 7 days after silk emergence. Ears were harvested approximately 63 days after midsilk, rated for insect damage, and shelled. The grain was ground and aflatoxin level determined. The transgenic corn hybrids sustained very little leaf feeding damage when infested in the whorl stage of growth with southwestern corn borer larvae and very little ear damage when infested at either stage of growth. The conventional hybrids sustained significant leaf and ear damage. The highest levels of aflatoxin were observed in the nontransgenic hybrids infested with southwestern corn borer after anthesis. The differences among aflatoxin levels were not, however, statistically significant. The low levels of aflatoxin for all treatments indicate that conditions were not conducive to aflatoxin accumulation. This makes defining the role that insect resistance could play in reducing aflatoxin accumulation in corn difficult.

COMPARISON OF PREHARVEST AFLATOXIN ACCUMULATION IN BT AND NON BT CORN IN FLORIDA AND GEORGIA

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Aflatoxin contamination assessments of temperate, tropical, Bt and non Bt corn were initiated in Florida in 1997 and in Bt versus non Bt corn in Georgia in 1998. There was little aflatoxin contamination seen in Florida in 1997 and no differences were observed in the planting date study at the North Florida Research and Education Center (NFREC) using strip tillage. In 1998 there was widespread preharvest aflatoxin contamination in corn in the southeastern United States. As a consequence the 1998 aflatoxin measurements were high in Florida and Georgia. Aflatoxin contamination means were highest in the first planting date of the Florida trials, followed by the second and third planting date trials. Seven hybrids were planted at the North Florida location on 27 March, 24 April and 22 May, 1998. The hybrids were Novartis N 79-P4, Novartis N 79-L3, Novartis N 7639 Bt, Pioneer 31B13 Bt, Pioneer 33Y09 Bt, Pioneer 32K61 and Pioneer 3098 (tropical). Grain yields were higher for most hybrids from the March 27 planting date than from corn planted later. The yields dropped significantly for the first three planting dates and for corn planted May 22 the yields were low. Aflatoxin content from corn planted March 27 was high and ranged from 250 to 815 ppb. There were no significant differences between hybrids. Aflatoxin content from the April 24 planting was high and ranged from 285 to 877 ppb. There were some significant differences between hybrids with one temperate hybrid being the highest and the tropical hybrid being the lowest in aflatoxin content. The three Bt hybrids were ranked 2, 5 and 6 in aflatoxin content and were not significantly different from each other. Aflatoxin content from the May 22 planting ranged from 16 to 952 ppb. However, the 952 ppb mean resulted from a single plot. There were significant differences in the log transformed data with the three Bt corns ranking 3, 4 and 5. In 1998,in Georgia there were no significant differences in aflatoxin content between Bt and non Bt hybrids in corn planted on 14 April, 12 May and 3 June in Plains GA or on three pairs of Bt and non Bt corn hybrids grown at Attapulgus, GA. In both Florida and Georgia the average aflatoxin concentrations were highest in the early planted corn and lowest in the late planted corn. The incorporation of the Bt gene in corn did not have a beneficial impact on aflatoxin content in the harvested product in Florida and Georgia.

OCCURRENCE OF FUMONISINS AND AFLATOXINS IN THE SOUTH GEORGIA CORN SURVEY FROM 1996 TO 1998.

Wellington Mubatanhema[1], Zeljko Jurjevic[1], David M. Wilson[1], Neil Widstrom[2], Filmore Meredith[3] and Bonnie Evans[1]; [1]University of Georgia, Tifton GA. [2]USDA/ARS, Tifton GA. [3]USDA/ARS, Athens GA

Corn samples were randomly collected before harvest from 41- 43 counties in south Georgia from 1996 to 1998. Analyses of fumonisins (FB_1+FB_2+FB_3) and aflatoxins (B_1+B_2+G_1+G_2) were carried out using HPLC and the Vicam aflatest fluorometer method respectively. The data was statistically analyzed using SAS. There was a high incidence of fumonisin contamination of corn from the three harvest seasons. In the 1996 samples, 86% of the corn samples had fumonisin concentrations ranging from 0.6µg/g to 30.3µg/g with four having concentrations between 0.6 and 1.0µg/g. In the 1997 samples 63% were contaminated with fumonisins, ranging from 1.0µg/g to 22.7µg/g. None of the contaminated samples had fumonisin concentrations below 1.0µg/g. In the 1998 samples 91% of the samples were contaminated with fumonisins, with concentrations ranging from 1.3µg/g to 33.3µg/g. None of the contaminated samples contained concentrations less than 1.0µg/g. All of the 1996 samples contained detectable amounts of aflatoxins, ranging from 5ng/g to 430ng/g with ten of the samples having an aflatoxin concentration less than 10ng/g. The 1997 samples on the other hand, had only 12% of the samples with aflatoxin concentrations above 10ng/g. Aflatoxin concentration in contaminated samples ranged from 1ng/g to 130ng/g. All the 1998 samples had aflatoxin contamination, with concentrations ranging from 6ng/g to 3500 ng/g with 95% of the samples having concentrations above 10ng/g. When data from all three years was combined, ear damage was correlated with both fumonisin and aflatoxin content and there was a significant positive correlation between fumonisin and aflatoxin contamination although this was not the case in the year by year analysis. The high incidence of fumonisin and aflatoxin contamination of south Georgia corn may pose economic and health risks.

FUNGAL SUCCESSIONS AND MYCOTOXINS OF STORED PEARL MILLET

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Pearl millet (*Pennisetum glaucum*) samples from 1996, 1997, and 1998 crop years were kept in experimental storage conditions to analyze fungal succession and mycotoxin development. The most frequently isolated fungi respectively were *Fusarium semitectum* (0-67%), *Fusarium chlamydosporum* (0-57%), *Alternaria spp* (0-28%), *Aspergillus flavus* (0-56%), *Curvularia spp.* (0-37%),and *Helminthosporium spp.* (0-30%). Less frequently found were *Aspergillus niger* (0-6%), *Aspergillus spp.* (0-4%), *Cladosporium herbarum* (0-7%), *Cerebella andropogonis* (0-9%), *Epicoccum spp.* (0-6%). *Fusarium verticillioides* (0-13%), *Gloeocercospora sorghi* (0-1%), *Penicillium spp.* (0-7%), *Phyllosticta spp.* (0-10%), *Phycomyces* (0-2%), *Rhizopus spp.* (0-8%), yeast (0-28%), and other fungi (0-4%). Aflatoxins were found in all three years in different amounts depending on year and storage conditions. Amounts ranged from 5 ng/g to 1750 ng/g. Highest amounts were detected in seeds from 1996 that contained approximately 20% moisture stored in 100% relative humidity at 25°C. Low levels of deoxynivalenol (0-0.3µg/g), nivalenol (0-0.4µg/g), and zearalenone (0-0.4µg/g) were detected from a standard panel of 35 different trichothecenes. No samples were positive for the presence of fumonisins.

PROFILE OF AFLATOXIN AND FUMONISIN ACCUMULATION IN NORTH CAROLINA CORN

Brian Bush, Marty Carson, Marc Cubeta, Winston Hagler, and Gary Payne; North Carolina State University, Raleigh, NC

Aflatoxin and Fumonisin are important contaminants in the North Carolina corn crop. Both mycotoxins are known to cause health problems in animals and limits are being placed on the amount of each toxin allowed in corn samples. The objective of this study was to determine the amount of Aflatoxin and Fumonisin occurring in North Carolina and the timing of infection corn kernels by each fungus. While the analysis of Aflatoxin and Fumonisin is not yet completed, the timing of infection has been determined. *Fusarium moniliforme* infected kernels first and reached a peak at 41.4% of kernels infected at sampling date 6 (9 weeks after silking). *Aspergillus flavus* infected kernels later and reached a peak at 2.7% of kernels infected at sampling date 9 (12 weeks after silking). Factors such as kernel moisture, rainfall data, insect feeding and the starburst symptom associated with *F. moniliforme* did not show a direct relationship with the number of kernels infected with *F. moniliforme* at this location. A relationship between these factors and F. moniliforme infection may become apparent once data are analyzed from our second location. Once the mycotoxin analyses are complete, we will have a profile of kernel infection and mycotoxin contamination of corn by these two fungi during the growing season.

POTENTIAL USE OF NATURAL PRODUCTS FOR PREVENTION OF FUNGAL INVASION AND/OR AFLATOXIN BIOSYNTHESIS IN CROPS

PANEL DISCUSSION

PANEL DISCUSSION TITLE: POTENTIAL USE OF NATURAL PRODUCTS FOR PREVENTION OF FUNGAL INVASION AND/OR AFLATOXIN BIOSYNTHESIS IN CROPS

PANEL MEMBERS: Bruce Campbell (Chair), Robert Norton, Nancy Keller, Charles Woloshuk and Gary Payne

SUMMARY OF PRESENTATIONS: This session chiefly focussed on natural products that effect the growth, development or biosynthesis of aflatoxin by aflatoxigenic *Aspergillus*. In this year's session, a number of speakers presented findings which indicated by-products of the metabolism of certain primary plant products, such as starches, sugars and fatty acids, could play a role in the regulation of aflatoxin biosynthesis or act as developmental signals to the fungus. Inhibition of the enzymes metabolizing these compounds could prove effective in reducing aflatoxin contamination. A role for the regulatory gene, *aflJ,* associated with the early stages of aflatoxin biosynthesis was also presented. In addition, use of genomic techniques to gain insights on genetic expression during host/fungal interactions was introduced as a promising future approach to better understand how to disrupt biosynthesis of aflatoxin.

Campbell (Campbell, Lee *et al.*) presented a brief overview of findings of the Plant Protection Research Unit at Albany, CA over the past five years; a period of time since scientists in that Unit were redirected to research aflatoxin contamination of tree nuts. He also presented new findings concerning efforts to use natural products for control of insect pests and *Aspergillus* species associated with the aflatoxin problem on tree nuts. The most significant findings over the past year included discovery of a volatile plant compound that acted as a lure to female codling moths, the major pest of walnuts whose feeding damage leads to infection by aflatoxigenic *Aspergillus*. Also, a number of natural compounds from edible plant sources were discovered that inhibit aflatoxin biosynthesis. These compounds show promise of inhibiting aflatoxin biosynthesis in processing streams. Lastly, further efforts to isolate the antiaflatoxigenic component of the 'Tulare' walnut were presented. This walnut variety shows almost complete inhibition of aflatoxin biosynthesis in laboratory tests. Findings on biotransformation of aflatoxin B1 by insects and anthocynanidin pigments in walnut seedcoats were also presented.

Norton presented results of efforts to find natural inhibitors of *Aspergillus* growth and aflatoxin biosynthesis in corn. A further question addressed in his research was to determine what changes occur when fungal growth invades from an area of a corn kernel which might have an inhibitory compound to an area which lacks an inhibitor. Using two glass-fiber disks mounted on a pin as a bioassay system, he was able to test the interplay of effects on aflatoxin biosynthesis by inhibitors included in one growth medium (lower disc) to eventual biosynthesis, if any, in a medium not containing an inhibitor (upper disc). Two inhibitors tested included a lipid, alpha-carotene, and an anthocyanidin, delphinidin. Both compounds inhibited synthesis in the lower disc over levels of inhibition in the upper disc. However, the results indicate that once aflatoxin biosynthesis was inhibited, mycelia from that fungal colony continued to show a reduced level of synthesis even after infecting media lacking an inhibitor. He also presented findings showing that certain simple sugars, such as talose, xylose and certain deoxy-glucoses, had an inhibitory effect on utilization of triglycerides for aflatoxin biosynthesis.

Keller presented further evidence that indicated derivatives of the metabolism of certain polyunsaturated fatty acids, namely linoleic acid, play a role in triggering morphogenesis of different developmental stages of aflatoxigenic *Aspergillus*. Previous research showed that derivatives of lipoxygenase activity on linoleic acid yielded factors, 13S-HPODE and 9S-HPODE, which affected

sporogenesis in fungi. Moreover, these compounds also affected levels of aflatoxin and sterigmatocystin biosynthesis in aflatoxigenic *Aspergillus*. To further gain insight on the effects of fatty acid metabolism on fungal development and aflatoxin biosynthesis, Keller showed how disruption of the desaturase that converts oleic acid to linoleic acid affects fungal morphogenesis and synthesis of sterigmatocystin. These findings were achieved by cloning functional and mutated, nonfunctional, forms of the odeA gene (oleic delta-12 desaturase) into *A nidulans*. The mutated strain lacked an ability to convert oleic acid to linoleic acid, showed abnormal conidial production and decreased sterigmatocystin levels. In the normal strain, odeA expression was greatest during conidiophore development.

Woloshuk provided further evidence linking the biodegradation of starch in *Aspergillus*-infected corn to biosynthesis of aflatoxin. He showed that alpha-amylase activity in an aflatoxigenic strain of *A. flavus* produced starch degradative products such as glucose, maltose and maltatriose, which elicited aflatoxigenesis. The importance of alpha-amylase to the production of aflatoxin was shown in that aflatoxin could only be produced if glucose was supplied to a mutant strain of *A. flavus* lacking alpha-amylase activity. Wounding of endosperm to embryo also facilitates aflatoxin production related to alpha-amylase activity. A trypsin inhibitor associated with resistance of corn to *Aspergillus* and aflatoxin production was also found to inhibit alpha-amylase, suggesting that its inhibitory properties may be associated with the inhibition of the degradation of starch. Since inhibition of alpha-amylase activity could prove to be a plausible strategy to reduce aflatoxin contamination, a search was conducted for anti-amylase peptides from a variety of sources. Of two hundred proteins screened, a highly active inhibitor was found in the legume, *Lablab purpureus.*

Payne presented further evidence on a role for the gene, *aflJ*. The role of this gene in the biosynthesis of aflatoxin had not been completely elucidated. This gene lies adjacent to *aflR* in the biosynthetic gene cluster for aflatoxin. He noted that disruption of *aflJ* resulted in reduced aflatoxin synthesis and reduction in certain intermediates within the pathway. A variety of transformation experiments with strains possessing and lacking either *aflR* and/or *aflJ*, were conducted to determine the role of *aflJ*. It was determined that *aflJ* appears to have no effect on transcription of certain genes early in the biosynthetic pathway, *ver-1*, and *omtA*. However, presence of *aflJ* did increase transcription of other genes in the biosynthetic pathway, namely *pksA* and *nor-1*. Thus, targeting this gene could serve as a means of preventing even the synthesis of early precursors of aflatoxin. Payne then discussed use of genomic techniques to better understand genes involved in regulation and synthesis of aflatoxin. He explained how he used a macroarray filter having a matrix made from a cDNA library of *A. flavus* to find what genes are expressed at different intervals during growth of *A. flavus*. and concurrent biosynthesis of aflatoxin. The macroarray experiments identified 89 differentially expressed genes between four and eight hours of growth. Some of these genes have been sequenced and are being compared to sequences in databases to infer their potential functional identity. The ultimate goal is to construct a matrix of these differentially expressed genes on microarrays to obtain better information on the sequential expression of genes during aflatoxin biosynthesis.

SUMMARY OF PANEL DISCUSSION: Merle Jacobs asked Bruce Campbell if aflatoxin had been found in any of the store-bought almonds after they had been placed on growth media and had shown contamination with *A. flavus* spores. Campbell explained that the fungi growing on the almonds was then grown separately on media. It was then determined whether that strain of *A. flavus* was aflatoxigenic. He stated approximately one out of every five almonds that showed fungal growth, possessed fungi that were aflatoxigenic. Themis Michailides asked why, in general, pistachios from the field have higher levels of aflatoxin than almonds. Campbell explained that this may have to do with a variety of conditions. One that may factor might be the level of insect damage, another important factor might be the particular variety of almond. Thirdly, the fact that

pistachios may be hydrated for a longer period of time in the field than almonds, could be a contributing factor. Campbell explained, according to research conducted by Unit scientist Noreen Mahoney, that on average walnut varieties showed the most inhibition, pistachios (of which only one variety is in commercial use, Kerman) is intermediate, and almonds varieties show least inhibition to aflatoxin biosynthesis. However, some almond varieties show inhibitory properties to aflatoxin production greater than Kerman pistachios. In any case these results were from laboratory conducted bioassays where all nuts were under a standardized treatment.

A question addressed to Gary Payne by Cheng Ji expressed some concern over the expense and cost-effectiveness of using microarrays or other genomics approaches. Gary explained that at this point in time, yes, genomic techniques are expensive, especially in the initial procurement of necessary hardware. But, he added, the costs are going down and there are now labs and other private facilities available who will perform some genomics-type services for a fee. Such costs on a piecemeal basis could prove to be cost effective without the large expense needed to establish an entire genomics facility. Peter Cotty asked if Gary had any ideas on how *aflJ* regulated transcription. Gary replied that the sequence of the gene product, thus far, was not predictive and that it could be that the gene product of *aflJ* might directly affect transcription or have an indirect effect by triggering the movement of an inducer that upregulates transcription.

Bob Norton was asked a number of questions concerning his bioassays. The question was asked why he used alpha- and not beta-carotene as a test inhibitor. Bob replied that in a previous study he found that alpha-carotene was six times more inhibitory than beta-carotene. He also stated that the bioassays were conducted in sealed scintillation vials and that the fungi were allowed to grow up to seven days. He placed the uninhibited discs in the upper position because he wanted to monitor effects on mycelia when the lower, inhibited, disc was removed at daily intervals. In addition, if the top disc was the one that was infected, the possibility existed that spores could have dropped down to the lower one by accident resulting in erroneous results with regard to "signaling" of inhibition of aflatoxin biosynthesis.

Hamad Abbas asked Nancy Keller if she had examined factors governing fumonisin production in *Fusarium*. Nancy responded that she had not. She was then asked how she was able to induce production of cleistothecia. Nancy explained that she was only able to obtain cleistothecia from *A. nidulans* and sclerotia from *A. flavus* and *A. parasiticus*. Nancy was asked if she found any *lox* (lipoxygenase) genes in *A. flavus*. She responded that she had, but only had preliminary characterization.

Jim Allen asked Charles Woloshuk if he had tried any proteinases to see if after treatment anti-amylase inhibitory activity was lost. He responded that he used proteinase K to knock out the inhibitory activity and that there was evidence that the inhibitor was a glycoprotein. He added that alpha-amylase was more temperature labile than the inhibitor.

Bruce Campbell commented that efforts to find natural product-based means of controlling aflatoxin contamination deserved more attention. This statement was made in view of potential problems in gaining acceptance of genetically modified foods (GMFs). He added that he did not intend to imply he had any misgivings with GMFs and that his statement was not meant to be detrimental in light of the diligent efforts of the genetically based approaches to control aflatoxin contamination.

PLATFORM PRESENTATIONS

TREE NUT-AFLATOXIN INTERACTIONS: NATURAL PRODUCTS AFFECTING INSECT PESTS, GROWTH OF *ASPERGILLUS*, AFLATOXIGENESIS AND AFLATOXIN BIOTRANSFORMATION

Bruce Campbell, Sung-Eun Lee, Douglas Light, Noreen Mahoney, Glory Merrill, Russell Molyneux, and James Roitman. USDA, ARS, Western Regional Research Center, Albany, CA

Research in the Plant Protection Research Unit focuses on reducing aflatoxin contamination of tree nuts, almond, pistachio and walnut. Almost all domestic commercial tree nuts are produced in California, where it has become a $2 billion/ year commodity. Moreover, over 50% of these tree nuts are exported, mainly to countries in the European Union (EU). Efforts to curtail the contamination of tree nuts in the Unit focus on two fundamental preharvest issues contributing to aflatoxin contamination. These issues are controlling insect feeding damage and growth of aflatoxin-producing *Aspergillus* or biosynthesis of aflatoxin. To achieve this control, methods are sought that are considered environmentally benign, that is they do not rely on use of procedures which might lead to further contamination of the tree nut ecosystem, surrounding agricultural community or indirectly impact nontarget species. In this regard scientists within the unit search for natural products of either the tree nut hosts, insect pests, or other plant sources that disrupt the normal biology of either the fungal or insect pests. In addition, there is another effort to find biocompetitive microorganisms that inhibit growth of aflatoxigenic *Aspergillus*. Because of the low tolerance thresholds set by the EU for aflatoxin (4 ppb) on imported commodities, the issue of aflatoxin contamination of tree nuts has become a trade as well as a food safety issue. Over the past year, several tons of US produced tree nuts were rejected by the EU. Thus, Unit scientists are also interested in finding ways of using antiaflatoxigenic natural products to prevent augmentation of aflatoxin production during the processing and shipping of tree nuts.

Insect feeding damage is one of the chief culprits leading to preharvest contamination of tree nuts. This damage breaches the protective layers of the nuts (hull, shell, seed coat) and allows spores of aflatoxigenic *Aspergillus* to germinate in the nut kernel. The main insect pests of tree nuts include the navel orangeworm (NOW), the codling moth (CM) and the peach twig borer (PTB). Research has mainly focused on identifying semiochemicals that disrupt the normal mating, host-locating, and feeding behavior of these pests. The ultimate goal is to apply effective semiochemicals in a manner that would significantly result in a decline in the insect pest population and/or reduction in preharvest feeding damage to tree nuts. To date, over 300 volatile compounds have been identified from the hosts of these insects pests. Over 150 of the compounds have been tested under field conditions. These tests mainly involve either attractancy or deterrency alone, or in combination with respective pheromones. Over ten volatiles have been identified that significantly increase (2 to 4-fold) the attractancy of pheromones. These compounds could be used in pheromone based mating disruption programs, or to improve trap-monitoring of pest populations. In addition, the pheromone constituents of PTB have been found to be more complicated than earlier findings. In some populations at least, the per cent composition of the two main components may vary considerably. This finding is significant from the standpoint that current programs using mating disruption to control PTB may have to reassess the components disseminated into orchards.

The latest finding by Unit scientist for control of tree nut insect pests include the discovery of a lure for female CM. This is a truly significant find in that it now provides some promise to development of a semiochemically based control of female CM. Current efforts rely chiefly on pheromones which are only attractive to male moths. However, it is the female moths that lay the eggs which eventually lead to feeding damage by emergent larvae. This lure has three-fold utility. Firstly, it provides a means of monitoring the gravid female CM population in pheromone based mating disruption

programs. Secondly, it can be used as a means of trapping female CM on a massive basis. And, thirdly, it can be disseminated in a mixture which includes a pesticide (so-called attracticide) to specifically target and kill only CM.

In addition to the semiochemical research. Unit scientists have taken a closer examination of the insect/ fungal/ tree nut interaction. It is common to find pest insects of tree nuts flourishing in nut kernels infected with aflatoxigenic *Aspergillus*. In view that aflatoxin is a common mutagen and carcinogen to many multicellular organisms, we are attempting to determine what enables these insects to survive, if not decontaminate aflatoxin. To date, NOW and CM have been examined. It was found that NOW exposed to aflatoxin B1 (AFB1) produce at least three or four biotransformation products. The main ones are aflatoxicol, aflatoxin B2a and aflatoxin M1, a fourth metabolite appears to be aflatoxicol M1. CM, both a field collected strain and a laboratory strain, appear to not metabolize AFB1 at all if only slightly, with formation of aflatoxicol. Glucuronide conjugates of these compounds were produced by both species. Of particular note, however, is that neither insect metabolizes AFB1 to aflatoxin B1-8,9 epoxide, the carcinogenic form that binds to DNA; which in humans leads to hepatocarcinogenesis. Unit scientists, have just isolated at least five anthocyanins in the seed coat of a "red" variety of the walnut *Juglans purpurea* developed by Gale McGranahan at UC Davis. Three of the aglycone anthocyanadins have been identified as malvidin, peonidin and petunidin. Robert Norton, an ARS chemist at NCAUR, has recently shown that certain anthocyanadins have anti-*Aspergillus* activity. However, the significance of such compounds in the seed coat of a walnut is that these compounds are antioxidants and could inhibit epoxidation of AFB1 in the liver.

Unit research has found a number of natural products of tree nuts that directly control fungal growth and aflatoxin production. These include anacardic acids and naphthoquinones in the hulls of pistachios and walnuts, respectively. In addition to these chemical constituents, significant differences between commercial and breeding varieties of both walnuts and almonds to support growth and or aflatoxin production have been found. This may be important for breeding resistance into tree nuts. In fact one variety of walnut, "Tulare" developed by Gale McGranahan is able to almost totally inhibit aflatoxin production. This is the only commodity known to date, among others affected by the issue of aflatoxin contamination, that exhibits this characteristic. Unit chemists are trying to isolate the chemical basis of this inhibition in this walnut variety. Thus far, they have found that it is not related to either amount or type of fatty acid content in the kernel. Unit chemists are also identifying natural compounds from a number of common food commodities that are potent inhibitors of *Aspergillus* growth or aflatoxin production. These compounds could have some utility in reducing potential of aflatoxin contamination (or contamination by other microbes of food safety interest) in processing streams. Because these compounds originate from common food sources their use may not fall under the strict guidelines set by the FDA for "food additives".

In addition to identifying natural products that may have utility in preventing aflatoxin contamination of tree nuts, another effort within the unit focuses on procuring microorganisms that could be used as biocontrol agents against aflatoxigenic *Aspergillus*. This effort mainly looks for microorganisms which already exist in tree nut orchards and are relatively safe to humans. A number of saprophytic yeasts have been identified that show significant inhibition against *Aspergillus*. Tree nuts treated with these yeasts show almost complete inhibition of growth of aflatoxigenic *Aspergillus* that inherently contaminate commercial products.

Future efforts by Unit scientists will be to:

1) Develop and implement CM control using the new lure.
2) Conduct a survey of California tree nut orchards to determine the strains of *Aspergillus* spp. present and their ability to produce aflatoxins.
3) Identify chemical basis of antiaflatoxigenic "Tulare" walnuts.
4) Examine use of identified natural product inhibitors of aflatoxin production in processing of tree nuts.
5) Continue to identify natural products affecting insect/ fungus/ tree nut interactions.

TRANSMISSION OF AFLATOXIN B_1 INHIBITION IN MYCELIA OF *A. FLAVUS* AND OTHER INHIBITOR STUDIES

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Corn germ is a major site for synthesis of aflatoxin B_1 (AFB_1) in infected kernels. If infection initially starts in the germ and then invades the endosperm it goes from a region with triglycerides as the principle carbon source to a region in which starch predominates. Much work is now being carried out to find inhibitors of *Aspergillus flavus* in corn, but the very distinct regions of the kernel make it less likely that if a suitable inhibitor is found that it will be expressed at a high level in both the endosperm and the germ. The question then, is what effect the presence of an inhibitor at the site of infection might have on aflatoxin production when the *A. flavus* spreads to a region of the kernel lacking the inhibitor. The suspended-disc system described previously was adapted to investigate this question. Two discs were mounted on a pin and separated by 3-3.5 mm. The lower disc contained medium and spores and either received inhibitor or served as a control. The upper disc received only medium. Growth and AFB_1 production in the upper disc was about 1 day behind that of the lower disc. Two inhibitors were used: α-carotene, a lipid, at 1 mg/ml and delphinidin, a charged water soluble flavonoid, at 5 mM (ca. 1.4 mg/ml).

Results: When both discs of the α-carotene-inhibited set were harvested at one day intervals the average AFB_1 inhibition, for days 4-7, was 77% for the lower and 81% for the upper discs. For days 5-8 inhibition was 83% for the lower and 72% for the upper discs. However for delphinidin inhibition was 99% for the lower and 59% for the upper discs. When the lower discs were removed at one day intervals (from day 2 to day 7) and the upper discs all harvested on day 7, average inhibition was 83% for lower and 72% for upper discs. Growth was not affected by either inhibitor in either set of discs. These results indicate that once the control discs are infected by inhibited mycelium removal of the inhibitor and infecting mycelia does not affect inhibition. α-Carotene inhibition was similar for both the infected discs containing inhibitor and those colonized by this mycelium but lacking inhibitor. Delphinidin showed a sharply lower level of inhibition in the secondarily infected disc indicating that the type of compound is important.

Previously we have shown that some sugars can inhibit the utilization of triglyceride for AFB_1 production by *A. flavus* NRRL 3357. The most active sugars were D-talose, 2-deoxy- and 6-deoxy-D-glucose and xylose. Dose/response curves were derived for these compounds: for talose the concentration for 50% AFB_1 inhibition (I_{50}) was 146 ug/ml. Talose inhibited growth with an I_{50} of 318 ug/ml. Inhibition was higher for 6-deoxy-glucose (I_{50} = 527 ug/ml) than for 2-deoxy-glucose (I_{50} = 1430 ug/ml). Xylose is a relatively common sugar and occurs in corn primarily as a component of cell walls. Its I_{50} was 1%. Mixtures of sucrose and xylose (4.5%:0.5%, 4:1, 3.5:1.5, 3:2, and 2.5:2.5, sucrose:xylose) were investigated in medium containing 10% corn oil to determine if the occurrence of xylose in the germ could affect AFB_1 level. Inhibition of 8-15% due to xylose was found in the mixtures. This compares with inhibition of 21% for 0.5% xylose to 92% for 4% xylose when it was the only sugar present with corn oil.

LINOLEIC ACID AND LINOLEIC ACID DERIVATIVES REGULATE *ASPERGILLUS* DEVELOPMENT AND MYCOTOXIN PRODUCTION

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Morphological development of *Aspergillus nidulans*, *Aspergillus flavus* and *Aspergillus parasiticus* is affected by linoleic acid (18:2) and linoleic acid seed-derivatives but not 18:1 or 18:0 fatty acids. The conversion of oleic acid (18:1) to linoleic acid is mediated by a delta-12 desaturase. We identified and cloned the delta-12 desaturase (*odeA*) in the model fungus *A. nidulans*. Analysis of the *odeA* nucleotide and putative amino acid sequence show characteristics similar to plant delta-12 desaturases. Chemical analysis of the *odeA* disruption strain (*odeA::argB*) showed that it is unable to produce linoleic acid but accumulates oleic acid. The *odeA::argB* strain presented defects in conidial production and mycelial growth rate as well as a decrease in sterigmatocystin production. In liquid shaken cultures, *odeA* is only detectable in the first 24 h of growth. However if the culture is allowed to develop in an air interphase, *odeA* is highly expressed during conidiophore development.

ALPHA AMYLASE FROM *ASPERGILLUS FLAVUS*: INHIBITORS FOUND IN PLANT SEEDS

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Our research over the past few years has focused on the identification metabolites in corn kernels that are involved in the induction of aflatoxin biosynthesis. We have suggested that these metabolites are susceptibility factors that are inherently part of the interaction between Aspergillus flavus and corn. Altering the production or the availability of these metabolites through methodologies such as breeding, chemicals, bioengineering or biocontrol would ultimately lower aflatoxin production.

Previously an aflatoxin-inducing activity was identified in culture filtrates of the aflatoxigenic A. flavus strain NRRL 3357 grown on maize kernels. The inducing activity was determined to be glucose, maltose, and maltotriose. We hypothesized that the source of these molecules was starch degraded by an amylase produced by A. flavus (1). An α-amylase-deficient mutant was isolated and assessed for its ability to infect and produce aflatoxin in wounded maize kernels. The α-amylase gene Amy1 was isolated from A. flavus and its DNA sequence was determined to be nearly identical to Amy3 of A. oryzae. When Amy1 was disrupted in an aflatoxigenic strain of A. flavus, the mutant failed to produce extracellular α-amylase and grew 45% the rate of the wild-type strain on starch medium. The mutant produced aflatoxin in medium containing glucose but not in a medium containing starch. The α-amylase-deficient mutant produced aflatoxin in maize kernels with wounded embryos and occasionally produced aflatoxin only in embryos of kernels with wounded endosperm. The mutant strain failed to produce aflatoxin when inoculated onto degermed kernels. In contrast, the wild-type strain produced aflatoxin in both the endosperm and embryo. These results suggest that α-amylase facilitates aflatoxin production and growth of A. flavus from a wound in the endosperm to the embryo.

A 14 kDa trypsin inhibitor associated with resistance to A. flavus and aflatoxin in maize was obtained from the research group of Robert Brown, USDA ARS SRRC. This protein also inhibited the α-amylase from A. flavus indicating that it is a bifunctional inhibitor. The inhibitor may have a role in resistance, limiting the growth of the fungus in the endosperm tissue by inhibiting the degradation of starch (2, 3).

Our data suggest that inhibiting the activity of the a-amylase produced by A. flavus may be an effective strategy for controlling aflatoxin production. We partially purified a-amylase from A. flavus to screen for amylase-inhibiting proteins. To screen for putative inhibitors, the α-amylase was incubated with protein extracts from various plant sources. Inhibition of α-amylase was determined as a decrease in the amount of reducing sugars released from starch added to the mixture. Two hundred protein extracts were tested and two inhibited α-amylase. The extract from the legume Lablab purpureus reduced alpha-amylase activity by 50%, whereas the extract from Sapindus drummondii inhibited the activity by 82%. Heating at 100 C for 10 minutes abolished the inhibitory activity (4). The inhibitor from L. purpureus was partially purified by ion-exchange chromatography, yielding a specific activity of more than 75 U/mg protein. A comparison with the 14 KDa inhibitor from corn suggest that the L. purpureus protein is 12 times more active. The inhibitor from L. purpureus was also active against the α-amylase from A. oryzae, but not the porcine pancreatic α-amylase (4).

References

1. Woloshuk, C. P., Cavaletto, J. R., and Cleveland, T. E. 1997. Phytopathology 87:164-169.
2. Fakhoury, A. M. and Woloshuk, C. P. 1999. Phytopathology 89: 908-914.
3. Chen,Z.-Y., Brown, R. L., Russin, J. S., Lax, A. R., and Cleveland, T. E. 1999. Phytopathology 89:902-907.
4. Fakhoury, A. M. and Woloshuk, C. P. 1999. Phytopathology 89: S25.

GENETIC STUDIES ON THE REGULATION OF AFLATOXIN ACCUMULATION

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The function of many genes in aflatoxin biosynthesis is known. One gene whose function in the pathway has remained elusive is *aflJ*. This gene resides within the aflatoxin biosynthetic cluster adjacent to the pathway specific regulatory gene *aflR*. Disruption of aflJ results in reduced aflatoxin biosynthesis and reduced conversion of metabolic intermediates. We examined the effect of *aflJ* on the transcription of the aflatoxin biosynthetic genes, *pksA*, *nor-1*, *ver-1*, and *omtA*, and on the accumulation of the early pathway intermediate, averantin. To conduct these experiments we used *Aspergillus flavus* strain 649-1, which lacks the entire aflatoxin pathway cluster. Transcription of *ver-1* and *omtA* was measured by monitoring GUS activity of the reporter constructs, *ver-1*::GUS and *omtA*::GUS. To ensure strong expression of *aflR* and *aflJ*, we used constructs of each in which their promoter was replaced the strong constitutive promoter, *gpdA*. To determine if the presence of *aflJ* leads to increased transcription of the pathway genes *ver-1* or *omtA*, 649 was transformed with either of these two reporter constructs along with either *gpdA::aflR* alone, *gpdA::aflJ* alone, or *gpdA::aflR* and *gpdA::aflJ* together. Transcription of either *ver-1* or *omtA* occurred only in the presence of *aflR* and the presence of *aflJ* did not increase their transcription. To determine if expression of *aflJ* leads to an increase in early pathway intermediates, strain 649-1 was transformed with cosmid 5E6 and either *gpdA::aflJ* alone, *gpdA::aflR* alone, or *aflJ* and *aflR* together. Cosmid 5E6 contains the genes *pksA*, *nor*-1, *fas-1*, and *fas-2*, that are required for the biosynthesis of the early pathway intermediate averantin. Transformants containing 5E6 alone produced no detectable averantin. In contrast, 5E6 transformants with *gpdA::aflR* produced averantin but only half as much as those transformants containing *aflR* and *aflJ*. Northern blot analysis showed that 5E6 transformants containing both *aflR* and *aflJ* had five times more *pksA* transcripts and four times more *nor-1* transcripts than 5E6 transformants containing *gpdA::aflR* alone. These data support the hypothesis that *aflJ* increases aflatoxin biosynthesis by increasing the transcription of the early pathway genes *pksA* and *nor-1*. Because of *aflJ* affects the regulation of the early steps in the pathway it is a prime target site for inhibition.

POSTER PRESENTATIONS

REPRESSION OF GUS REPORTER CONSTRUCTS OF THE AFLATOXIN BIOSYNTHETIC PATHWAY GENES BY PHENOLIC COMPOUNDS

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One unique characteristic of plant-microbial interactions is that plant-associated bacteria or fungi respond to phenolic signal molecules. Acetosyringone, identified as a phenolic signal molecule in *Agrobacterium tumefaciens*-plant interactions, was shown to affect virulence *vir* (virulence) gene expression. In *Pseudomonas syringae*, certain phenolic compounds were demonstrated to activate syringomycin biosynthetic gene expression (*syr*B and *syr*D). It was shown that phenolics on fruit surfaces inhibited cutinase production in *Monilinia fructicola.*

In searching for natural compounds inhibitory to aflatoxin biosynthesis we found that the phenolic molecules acetosyringone, syringaldehyde and sinapinic acid reduced the biosynthesis of aflatoxin B_1 (AFB_1) in *A. flavus*. Among the three compounds acetosyringone was the most active one. These phenolics also inhibited the formation of norsolorinic acid, an early intermediate of the AFB_1 biosynthetic pathway. We then used GUS (b-glucuronidase) reproter gene constructs to demonstrate that acetosyringone repressed *nor1* and *ver1* expression equally well in fungal cultures grown in GMS. Both genes were coordinately induced in GMS and repressed by acetosyringone in a concentration dependent manner in two GUS reporter gene constructs.

The expression of *nor* and *ver* genes is dependent on *aflR* , a pathway- specific transcriptional activator. These phenolics may act by affecting the biosynthesis of AFLR (activator protein). Another possible mechanism by which acetosyringone could affect aflatoxin biosynthesis is that the compound binds to AFLR protein so it can no longer serve as an efficient activator for the expression of the aflatoxin biosynthetic genes.

Two reporter genes of *aflR* have been constructed recently, based on GUS and GFP. The jellyfish green fluorescent protein, GFP, in a reporter construct is an excellent tool to determine the effect of acetosyringone on the biosynthesis of the AF pathway activator. Since GFP is very stable, research is in progress to extract this protein from fungal cultures for determining its concentration. Conditions to visualize GFP by confocal scanning microscope and fluorescence microscope in a *aflR::gfp* transformant of *A. flavus* are being optimized.

By gaining an understanding of the molecular mechanisms leading to the expression or repression of AF pathway genes, it should be possible to design rational strategies for the control of aflatoxin production. Identification of plant metabolites which alter the expression of AF genes should contribute to the elimination of aflatoxin contamination in agricultural commodities.

ISOFORM PATTERNS OF CHITINASE AND β-1, 3-GLUCANASE IN MATURING CORN KERNELS (*ZEA MAYS* L.) ASSOCIATED WITH *ASPERGILLUS FLAVUS* MILK STAGE INFECTION

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Isoform patterns of chitinase and β-1, 3-glucanase of maturing kernels of yellow corn infected with *Aspergillus flavus* were investigated through polyacrylamide gel electrophoreses (PAGE). Proteins on the SDS gel with an apparent molecular weight range of 23-46 kDa were induced in the kernels by *A. flavus* infection. From in-gel (native PAGE) enzyme activity assays, three bands corresponding to chitinase isoforms and two bands corresponding to β-1, 3-glucanase isoforms were detected in the infected kernels. One chitinase isoform of 29 kDa was induced only in the infected kernels, and another one of 28 kDa was present in both infected and non-infected kernels. They were both acidic based on their migration on an acrylamide isoelectric focusing (IEF) gel. For the β-1, 3-glucanase, one isoform of 35 kDa was present in both infected and non-infected kernels, but another one, 33 kDa isoform, was induced only in the infected kernels. Both acidic and basic β-1, 3-glucanase isoforms were detected in the IEF gel. The results indicate that only particular isoforms of the two fungal degradative enzymes might be involved in the maturing corn kernels infected at the milk stage with *A. flavus*.

EFFECTS OF A NOVEL COMPOUND ON EXPRESSION OF MYCOTOXIN BIOSYNTHETIC GENES

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Abstract

Mycotoxins are fungal secondary metabolites that contaminate food and feed and are toxic to animals. Several of the mycotoxins are of public health concern including aflatoxin, ergot alkaloids, ochratoxin, fumonisins, deoxynivalenol, and T-2 toxin. In many cases, fungi that produce mycotoxins invade crops before harvest and are likely to proliferate during storage under favorable conditions. Control of mycotoxin contamination has been difficult and novel approaches towards their elimination are sought. Since the majority of the damage done by many of these fungi is due to mycotoxin contamination, and traditional means of elimination of the fungi have been unsuccessful, we have been screening for compounds that affect the regulation of genes involved in mycotoxin production. Finding natural compounds that affect the toxin-producing processes in mycotoxigenic fungi is an important step toward the generation of new control strategies. We have developed a thin-layer-chromatography (TLC)-based assay to use genetically engineered isolates of *A. parasiticus* to screen for compounds that have inhibitory effects on the expression of aflatoxin genes. This assay was used to track purification of an active compound, called cp2, from black pepper, *Piper nigrum*. The objective of this research was to determine the range of biological activity of cp2. Cp2 inhibits transcription of aflatoxin biosynthetic genes in *A. parasiticus*, but does not affect mycelial growth. Cp2 also affects the transcription of genes of secondary metabolism for *A. nidulans* (ipn) and *Gibberella zeae* (tri5). Once final structure determination of cp2 has been completed, analogues will be synthesized to determine the active moieties of the compound. In addition, studies have been initiated to determine biosynthesis of cp2 in *Piper nigrum*. These studies will be essential to final determination of the feasibility to use of Cp2 to engineer mycotoxin resistant crops.

ADVANCES TOWARD DEVELOPMENT OF A HOST PLANT VOLATILE-BASED ATTRACTICIDE FOR CODLING MOTHS, A KEY PEST IN *ASPERGILLUS* INVASION OF WALNUTS

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The codling moth (CM) is the key pest of walnuts, damaging the husk, shell, seed-coat and kernal of walnuts thereby allowing for the invasion and colonization by *Aspergillus* species and the production of aflatoxin. Last year, we reported the discovery of a new, host-plant volatile - derived "HPV #23," bisexual attractant for the codling moth. This attractant, "HPV #23," is the first Lepidopteran bisexual lure, specific for both females and males, that is a single compound and has potency and attractancy properties comparable to sex pheromones attraction of exclusively males.

This year we have made progress in defining and developing this lure/attractant for its practical control uses through strides in team organization and technology transfer, behavioral analysis, practical use assessment, and application of evolving control technologies.

First, our ARS Unit's potential and capabilities to achieve implementation success have greatly been enhanced by the formation of a cooperative research endeavor (CRADA) with Trécé, Inc., the world's leader in insect semiochemical traps and monitoring systems. The week of this meeting, our CRADA team is filing a patent application on the discovery of this novel and powerful attractant and its applied uses in controlling this world-wide pest. Due to patent infringement and propriety concerns, we will not divulge the chemical structure of the attractant and its related compounds, and will simply refer to it as, "HPV #23."

Second, we have had success in rigorously determining the "active ingredient" for this CM-HPV attractant through selectivity and structural specificity tests on the attractancy of synthesized and highly pure chemical analogs. Further, we have determined that CM are highly sensitive to the "HPV #23" attractant, which was supported also by the strong potency and longevity evidence for this lure.

Third, we have demonstrated, in both conventional orchards and mating disruption orchards, the practical use of this CM-HPV attractant as a tool that is as accurate as pheromone for detecting moth emergence and delineating flight patterns but defines not just the males but also the females population trends. It is the presence and egg-laying of female moths that determines the damage potential of the next larval feeding generation, and thus the need for and appropriate timing of control measures.

Fourth, we have initiated the design and testing of attracticide formulations that slowly and effectively release the "HPV #23" attractant and effectively poison on short contact the attracted moths.

These advances all point to the future promise of implementing this developing novel control technology for the effective protection of walnuts from CM damage and *Aspergillus* infection.

BIOTRANSFORMATION OF AFB_1 IN NAVEL ORANGEWORM AND CODLING MOTH LARVAE

Sung-Eun Lee, Douglas M. Light and Bruce C. Campbell. USDA, ARS, Western Regional Research Center, Albany, CA

The navel orangeworm (NOW), *Amyelois transitella* (Walker), is a major pest of almonds and pistachios and the codling moth (CM), *Cydia pomonella* L., is the principal pest of walnuts. Feeding damage by their larvae creates wounds in the protective layers (*i.e.*, hull, shell and seed coat) that surround the nut kernel. These wounds allow spores of the aflatoxin producing fungus, *Aspergillus flavus*, to infect nuts. This frequently results in a microenvironment where the insect is in contact with *A. flavus* and, therefore, aflatoxin. Interestingly, these two insect pests can survive in nuts severely infected by the fungus. However, little is known about aflatoxin metabolism by insects including these two insects, even though chronic and acute toxicity and mutagenicity of AFB_1 to insects have been reported.

Aflatoxin metabolism of AFB_1 in NOW and CM was compared to that of mice and chicken. A field strain of NOW produced three metabolites and these metabolites were identified as aflatoxicol (AFL), aflatoxin B_{2a} (AFB_{2a}) and aflatoxin M_1 (AFM_1). This strain of NOW produced mostly AFL and small amounts of AFB_{2a} and AFM_1. Metabolism of AFB_1 by chicken liver also produced mostly AFL, while mouse liver showed an 8-fold production of AFM_1 over AFL. Interestingly, a lab strain of CM larvae did not produce any metabolite *in vitro*, whilst a field strain of the insect showed a low production of AFL, only.

Metabolism of AFL by NOW larvae yielded two metabolites identified as AFB_1 and presumably aflatoxicol M_1 ($AFLM_1$). This finding showed interconversion between AFB_1 and AFL. This interconversion may prolong exposure of insects to AFB_1.

The differences between NOW and CM larvae in the biotransformation of AFB_1 was highly significant. However, neither insect produced AFB_1-8,9-epoxide, a metabolite of AFB_1 which binds to DNA and believed to be responsible of the carcinogenic effects observed in many organisms exposed to aflatoxin. Further studies are being conducted on the differences between these insects in their metabolism of aflatoxin. But, to date, at least three biotransformtion pathways are identified in NOW.

NATURALLY ACTIVE COMPOUNDS AS INHIBITORS OF AFLATOXIN BIOSYNTHESIS

Sung-Eun Lee, Noreen E. Mahoney and Bruce C. Campbell. USDA, ARS, Western Regional Research Center, Albany, CA

Aflatoxins are tasteless, odorless and colorless mycotoxins, which are secondary metabolites produced by certain strains of *Aspergillus* spp., and are known to be mutagenic, carcinogenic and teratogenic to most animals and humans. Thus, contamination of agricultural commodities such as tree nuts including walnut, almond and pistachio, by aflatoxins is a public health concern. Moreover, efforts to control aflatoxin-producing *Aspergillus* with fungicides or antibiotics are not environmentally or economically sound at this point. Therefore, there is a need to find alternatives for antibiotics and fungicides to inhibit aflatoxin during pre- or postharvest processing. In this regard, we have initiated an effort to find potent inhibitors of aflatoxin-producing *Aspergillus* that are natural products of edible commodities.

Dried fruit of a common Asian plant was tested for its inhibitory activity against growth and aflatoxin production of *Aspergillus flavus*. The dried fruit inhibited aflatoxin production at a concentration of 0.50 mg/mL after 7 days of incubation. However, the growth of *A. flavus* was moderately inhibited by the dried fruit. Recently isolated compounds from this dried fruit were also tested for their inhibitory activities against *A. flavus*. One of the tested compounds showed a potent inhibitory activity on aflatoxin production as complete control was found at the concentration of 0.2 mg/mL. This compound also showed moderate inhibition on growth of *A. flavus*. These compounds are safe for human consumption and could be useful to inhibit aflatoxin production during processing and shipping of food commodities.

EFFECT OF ALMOND PROCESSING ON VIABILITY OF *A. FLAVUS* SPORES

N. E. Mahoney, R. J. Molyneux, P. Bayman; USDA, ARS, Western Regional Research Center, Albany, CA

Two-thirds of the California almond crop is exported, with a considerable percentage going to the European Union (EU). New regulations imposed by the EU limit aflatoxin in foods to no more than 4ppb total aflatoxin and 2ppb aflatoxin B1. The California almond industry is committed to complying with this strict new standard. One area of concern is manufacturing grade almonds, which usually have some type of preharvest insect or physical damage. These almonds are blanched in a 180 to 212°F bath, followed by removal of the seed coats. The nuts are then dried and chopped, slivered, or sliced. The concern with this processing method is that it may increase the susceptibility of these nuts to postharvest aflatoxin contamination. Moisture from the blanching process could stimulate fungal activity from existing mycelia or the germination of new *Aspergillus flavus* spores. These nuts also may be more susceptible to fungal colonization because the seed coats, which act as a barrier to fungal infection, are removed, and because they already contain areas of damage, which are conducive to *A. flavus* germination and growth.

We tested the effect of temperature on the viability *A. flavus* conidia to determine if spores could survive the blanching process and potentially contribute to postharvest aflatoxin contamination. There was no reduction in viability in spores kept up to 30min. at 120 to 130°F, compared with 72°F. However; the number of colonies was higher at 120°F than 72°F for all incubation times, indicating that spore germination may be stimulated by slightly elevated temperatures. At temperatures below 140°F, spores germinated by 48hrs and no additional colonies developed after this time. A reduction in spore viability was evident after 5min. at 140°F. Colonies were significantly fewer and took longer to develop. After 10 and 15min. at 140°F *A. flavus* colonies were slower to develop and the germination time for some spores increased to 3 or 4 days. A complete loss of spore viability occurred after 30min. at 140°F and after 5 min. at 150°F, with no colonies visible after 7 days of incubation.

Therefore, *A. flavus* spores are vulnerable at temperatures well below the 180 to 212°F used in the blanching process; and blanching may reduce the potential for postharvest aflatoxin contamination in manufacturing grade almonds. There is also the potential for reduction of postharvest contamination in pistachios by using heat above 140°F in the flotation bath used during processing.

APPROACHES TO A NAVEL ORANGEWORM BIOASSAY FOR ASSESSING ANTIFEEDANT PROPERTIES OF NATURAL PRODUCTS

James N. Roitman; USDA-ARS-Western Regional Research Center, Albany, CA

Feeding by larvae of the navel orangeworm (NOW), a major insect pest of walnuts and almonds, is a primary cause of fungal spore contamination of immature nuts. When temperature and moisture conditions favor spore germination and fungal growth, aflatoxigenic *Aspergillus* species grow vigorously, often producing high levels of aflatoxin. Earlier studies carried out at WRRC on the effects of flavones on navel orangeworm larvae suggested that several of these naturally occurring substances were effective antifeedants at low concentration and that they might be useful in reducing NOW damage to nuts. The role of flavonoids in plants has not been rigorously established although it has been assumed that they provide protection from the damaging effects of intense solar ultraviolet radiation, from predation by insects and from invasions by microorganisms. We decided to expand the earlier study by developing an artificial NOW diet based on almonds in order to bioassay flavones and other natural products. Two approaches to developing a bioassay were developed, one using whole almonds as the sole food source, the other using ground almond powder mixed with agar.

1. A small area of skin was scraped from each whole raw almond to ensure that the neonate navel orangeworm larvae would have easy access to the nutmeat. Nuts were then rehydrated by autoclaving with enough water to raise the moisture level to 20%, dipped in acetone solutions of substances to be tested and the solvent allow to evaporate for several minutes. The nuts were placed individually into small plastic cups, two neonate larvae transferred to the scarred area of each nut and the cups closed with lids punctured with two small holes and sealed with parafilm allowing air diffusion but preventing desiccation. The cups were placed in a growth chamber maintained at 25-27° for 14 days under a 14hr/10hr light/dark regimen, then surviving larvae weighed. Results were disappointing because of 20 control cups having no added substances only 14 had living larvae and these had very low weights (average 800 micrograms) weights of 30 – 60 mg. had been anticipated.

2. Commercial almonds were ground in a coffee mill, sieved through a 20 mesh screen and autoclaved after 1.3% methyl *p*-hydroxybenzoate had been added. Test substances were absorbed onto a small amount of a-cellulose from acetone solutions, the acetone evaporated, almond powder added and freshly autoclaved 2% agar added with thorough stirring. After gelling, the agar was cut into 10 equal pieces and each was placed in a plastic cup with two neonate NOW larvae. The cups were sealed as described above and held for 14 days at 25-27° under a 14hr/10hr light/dark regimen. Cups were inspected under a dissecting microscope for signs of larval activity and living larvae were weighed. Results were also disappointing with low larval survival rates both on the control and treated diets and weights of survivors averaged only 2mg.

Although neither of the approaches described above was successful the powdered almond/agar diet has several advantages. The concentrations of test substances in the diet can be accurately controlled and additional nutrients can easily be added. We intend to make some modifications adding nutrients to see if the 14 day survival rate and larval weights can be increased to values approximating those encountered in the field (30-60 mg.).

COMPARISON OF AFLATOXIN PRODUCTION ON DEFATTED TREE NUTS HIGH ANTI-AFLATOXIGENIC ACTIVITY IN WALNUT

N. E. Mahoney[1], R. J. Molyneux[1], B. C. Campbell[1], G. H. McGranahan[2], and T. M. Gradziel[2], [1]USDA, ARS, Western Regional Research Center, Albany, CA; [2]Deparment of Pomology, University of California, Davis, CA

We are examining varietal diversity as a source of natural resistance to aflatoxin production in tree nuts in order to reduce the high levels of aflatoxin found in a small percentage of nuts that could cause contamination of an entire shipment. The pistachio variety Kerman accounts for the majority of commercial pistachios; however, both almonds and walnuts have many varieties in commercial production. The overall average aflatoxin produced on the 26 walnut varieties tested was almost 10 times lower than the aflatoxin produced on the Kerman pistachio, and almost 25 times lower than the average aflatoxin produced on the 34 almond varieties tested. The walnut variety Tulare was unique among all the tree nut varieties tested in that, while it did support fungal growth, only trace levels of aflatoxin were produced.

One of the major differences between walnuts and the other tree nuts is fat content. The average fat content of walnuts is about 70%, compared with between 40 and 50% for almonds and pistachios. The fat composition of walnuts is also significantly different than the fat composition of pistachios and almonds. The fat in almonds and pistachios is primarily composed of the monounsaturated fatty acid oleic acid, while walnut fat is primarily composed of the polyunsaturated fatty acids linoleic and linolenic acids. Unsaturated triglycerides affected aflatoxin production depending on the level of unsaturation. Trilinolein added as the sole carbon source for *A. flavus* resulted in half the aflatoxin produced on triolein. Trilinolenin did not support any aflatoxin production.

While fat composition may contribute to the low aflatoxin produced on walnut varieties, defatted Tulare meal has significant aflatoxin inhibitory properties. The defatted meals from Kapareil almond, Mission almond, and Kerman pistachio significantly increased aflatoxin production compared with the control. The defatted meal from Chico walnut did not affect aflatoxin production compared with the control. The defatted meal from Tulare walnut was inhibitory, resulting in 79% lower aflatoxin production compared with the control.

CHARACTERIZATION OF THE DELTA-12 DESATURASE GENE OF *ASPERGILLUS NIDULANS* AND ITS RELATIONSHIP WITH SPORE DEVELOPMENT AND STERIGMATOCYSTIN PRODUCTION

Ana Calvo[1], Harold Gardner[2], and Nancy Keller[1]; [1]Texas A&M University, College Station, TX and [2]USDA/ARS/NCAUR, Peoria, IL

Morphological development of *Aspergillus nidulans*, *Aspergillus flavus* and *Aspergillus parasiticus* is affected by linoleic acid (18:2) and linoleic acid seed-derivatives but not 18:1 or 18:0 fatty acids. The conversion of oleic acid (18:1) to linoleic acid is mediated by a delta-12 desaturase. We identified and cloned the delta-12 desaturase (*odeA*) in the model fungus *A. nidulans*. Analysis of the *odeA* nucleotide and putative amino acid sequence show characteristics similar to plant delta-12 desaturases. Chemical analysis of the *odeA* disruption strain (*odeA::argB*) showed that it is unable to produce linoleic acid but accumulates oleic acid. The *odeA::argB* strain presented defects in conidial production and mycelial growth rate as well as a decrease in sterigmatocystin production. In liquid shaken cultures, *odeA* is only detectable in the first 24 h of growth. However if the culture is allowed to develop in an air interphase, *odeA* is highly expressed during conidiophore development.

CROP RESISTANCE - GENETIC ENGINEERING

PANEL DISCUSSION

PANEL DISCUSSION TITLE: CROP RESISTANCE – GENETIC ENGINEERING

PANEL MEMBERS: K. Rajasekaran (Panel Chair), Paul Hasegawa, Anne-Laure Moyne, Gale McGranahan, Arthur Weissinger, Peggy Ozias-Akins, Caryl Chlan.

SUMMARY OF PRESENTATIONS: Preliminary but promising results on potential uses of antifungal peptides and proteins in transgenic cotton and peanuts were presented in this years meeting following the demonstration last year of the utility of these gene constructs in control of *A. flavus* using tobacco model system. The anti *A. flavus* properties of osmotin, produced by plants in response to pathogen attack, and a newly identified peptide from *Bacillus subtilis* were presented at the meeting. Results on genetic engineering and conventional breeding approaches to enhance resistance to *A. flavus* in walnut were also summarized.

Mike Hasegawa and his colleagues (Purdue University) reported on the mode of action of osmotin, a defensive protein produced by plants in response to invading pathogens. He reported, based on yeast model system, that osmotin activates a G-protein response in *A. nidulans* that facilitates its access to the plasma membrane, the site of antifungal action. Thus, identification of *A. flavus* vulnerability to osmotin and other PR proteins (e.g., wheat PR-4) could be exploited in transgenic crop plants.

Dr. Anne-Laure Moyne, working with Dr. Sadik Tazun of Auburn University reported on isolation of a strain of *Bacillus subtilis* AU195 that produces two small antifungal peptides. These two peptides are very stable and with a broad range of activity against fungi and a very high antifungal activity against *A. flavus*. Further analysis indicated that these peptides are cyclic and belong to the iturin family and with the same amino acid sequence as the bacillomycin D.

The USDA, SRRC group (Rajasekaran et al.) reported on a novel method of assaying putative transgenic cottonseed for *A. flavus* resistance using a GFP-expressing strain. They observed that immature seeds from some of the putative R_0 transgenic cotton plants showed reduction in *A. flavus* growth in situ, measured as GFP fluorescence emanating from the invading fungus. Cottonseed extracts from these plants also inhibited growth of *Verticillium dahliae*. Identification and characterization of plants that are homozygous to the trait in the R_2 progeny through molecular and antifungal assays will be attempted in the coming year.

Dr. Caryl Chlan (University of Louisiana at Lafayette) working in conjunction with the SRRC group reported on the efficacy of several gene constructs such as osmotin, lytic peptides, polygalacturonase inhibitor, haloperoxidase, and basic bean chitinase/glucanase in transgenic *Arabidopsis* plants. She reported that one of the most effective gene constructs was the haloperoxidase construct based on *in planta* assays with a natural *Arabidopsis* pathogen, *Pseudomonas syringae* pv. tomato. She also reported on a cottonseed specific promoter (CSSP) and a defense gene promoter for tissue specific expression in seeds.

Dr. Art Weissinger (North Carolina State University) reported on the progress with transgenic peanut and tobacco plants carrying the gene that codes for the antifungal peptide, D5C. Transgenic tobacco plants showed delayed symptom development following inoculation with *Rhizoctonia* and *Phytophthora*. Similarly, some R_0 transgenic peanut plants showed a good correlation between the presence of the D5C transgene and resistance to *Cercospora arachidicola*. However, analysis of R_1 peanut progeny plants did not show statistically significant difference between transgenic progeny and control plants. Subsequent analyses indicated two problems in the transformation experiments -

and control plants. Subsequent analyses indicated two problems in the transformation experiments - homology-dependent silencing and the possible phytotoxicity of the constitutively expressed peptides, which might reduce recovery of transgenic peanut plants.

Dr. Peggy Ozias-Akins' group at Tifton, GA has continued evaluation of transgenic peanut lines expressing *CryIA*(c) as a possible means of inhibiting *A. flavus* inoculation into peanut pods by lesser cornstalk borer. Southern analysis revealed that progenies of insect-resistant lines were derived from at least two independent transformation events. Field trials with the R_6 generation and measurements for aflatoxin levels have been planned for the coming year. In addition, she presented details on other gene constructs such as tomato anionic peroxidase, antifungal peptide D4E1, and soybean lipoxigenase, that have been employed in transformation of peanuts in her laboratory.

All the three labs working on antifungal peptides (Rajasekaran, Weissinger and Ozias-Akins) highlighted the importance of characterization of transgenic plants at the protein level and the need for a suitable detection method, either by immunological or mass-spectrometry method.

Dr. Gale McGranahan (University of California, Davis) reported on her work with Dr. Abhaya Dandekar (UCD) and others on transformation of walnut with antifungal genes. One hundred and fourteen transgenic walnut plants carrying the gene for *CryIA*(c) have been planted in two locations, Davis and Fresno, CA, for evaluation of damage by codling moth larvae and subsequent onset of *A. flavus* infection. She also reported on identification of a full-length polyphenol oxidase cDNA from walnut embryos which is being tested for its antifungal or anti-insect activity. In addition, she reported that evaluation of 26 walnut cultivars, representing a range of germplasm at WRRC, has resulted in identification of one variety, Tulare, for its consistent low aflatoxin content. The value of the intact seedcoat in preventing aflatoxin problem is being addressed in crossing experiments involving Tualre (a low aflatoxin producer) and Hartley (a high aflatoxin producer).

SUMMARY OF PANEL DISCUSSION: Gale McGranahan was asked if the aflatoxin problem is over in walnut production following the release of cv. Tulare. She confirmed that cv. Tulare is not only resistant to aflatoxin contamination but also a high yielding variety. This variety is replacing the currently popular cv. Chandler and is being currently used in grafting of existing cultivars.

Themis Michailides commented regarding the presentation by Rajasekaran by saying that partial (up to 60%) reduction in *A. flavus* growth in immature cottonseed from putative cotton transformants is not adequate from plant pathology point of view. Rajasekaran replied that the results are from a segregating R_1 population and further evaluation of R_2 progeny plants, that are homozygous for this trait, is necessary for proper conclusion; In addition, the transgenic control is not comparable to chemical control, where total control of a pathogen is targeted. Art Weissinger added that 60% reduction in *A. flavus* growth is very significant considering the effect of a single gene. It was agreed that multiple approaches are required to combat the aflatoxin problem.

John Radin asked Art Weissinger if peptides could be evaluated early on for negative effects on animals and humans. Art stated that these peptides are cleaved by proteases. He also mentioned that the peptide D5C has thrombin cleavage sites and gets disassembled in human blood. To an additional question by John Radin on the effect of peptides on rhizosphere bacteria or endophytes, Art replied that there is no data available as yet. Mike Hasegawa added that the real target is plasma membrane and toxic molecules are usually highly specific for cell types.

Abbas asked Anne-Laure Moyne regarding toxicity of two peptides from *Bacillus subtilis* to humans. She answered they are not toxic to humans. She also mentioned that the stability of these cyclic

peptides is not known. To another follow-up question, she answered that it is not effective against *Fusarium moniliforme*. She added that the gene for the cyclic peptides are too large to be of use in genetic engineering of crop plants but the bacterium might be a good candidate for use as a biocontrol agent.

Corley Holbrook asked Art Weissinger if greenhouse assay results correlate with field assay with respect to preharvest aflatoxin contamination and asked him to comment on the applicability of *in vitro* techniques. Art Weissinger answered that field assays using the GFP-*A. flavus*, as reported by Keith Ingram are extremely useful to arrive at meaningful results. Art mentioned that the phytotoxicity of D5C limited the recovery of transgenic peanuts in his laboratory. Dave Wilson commented that bioassays for *A. flavus* inhibition need to be looked at more closely for further possible refinement. He suggested that development of an assay similar to corn kernal screening assay might be useful.

Francis Trail asked the panel if suitable promoters have been identified for use under drought stress conditions since drought stress-activated promoters will be useful in aflatoxin prevention program. In response to his question, Mike Hasegawa replied that drought stress genes have PR-type and ABA- responsive elements on these promoters. Osmotin, he added, is a PR5 family member and exhibits all these functions including upregulation by drought. The Peanut Foundation is interested in this osmotin promoter and has paid for it. Art Weissinger asked Mike Hasegawa if osmotin promoter is active enough under non-drought conditions. Hasegawa replied that it is not and its expression is less than that of a constitutive promoter and the most active portion of osmotin is equal to that of Gelvin's superpromoter. However, it is inducible by ABA and, therefore, drought. It was commented that less expression of antifungal proteins would mean development of resistance among pathogens. John Radin commented that according to EPA regulations, at least 25 times the threshold levels of lethal dose, as in the case of Bt transgenics, is necessary for approval and proper release of a transgenic variety.

Jay Mellon asked Peggy Ozias-Akins if native peanut promoters could be used to avoid transcriptional silencing. Peggy replied that multiple copies of a peanut promoter might lead to silencing as well. Nancy Keller asked Peggy if one could use transient assays to look at silencing problem. Peggy replied that this phenomenon probably could not be studied in a transient assay. Art Weissinger added that at times silencing takes place after several generations and emphasized the need for more work to understand the phenomenon. Don White concluded by saying that even the discards among progenies need to be looked at more carefully to understand the mechanisms of failure with regard to gene expression.

PLATFORM PRESENTATIONS

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WHEAT PATHOGENESIS-RELATED (PR)-4 PROTEIN AND OSMOTIN CONTROL OF *ASPERGILLUS* GROWTH

Bressan, R. A., Coca, M., Narasimhan, M., Damsz, B., Veronese, P., Huh, G.-H., Salzman, R., Hasegawa, P. M.; Center for Plant Environmental Stress Physiology, Purdue University, West Lafayette, IN

The goal of this project is identification of *Aspergillus flavus* vulnerabilities to plant defensive PR proteins that can be exploited for the control of growth and aflatoxin production by the fungus. The first research objective is to establish a recombinant protein expression system for production of sufficient quantities of active wheat PR-4 that can be used for evaluation of efficacy and characterization of function. The second objective is further molecular genetic dissection of osmotin-mediated growth inhibition of *A. nidulans* as a paradigm for plant defense against *A. flavus*. Progress pertaining to the first objectives includes the isolation of a cDNA clone that encodes a full length wheat PR-4 protein. Bacterially expressed recombinant protein accumulates primarily in inclusion bodies regardless of the vector employed, and solubilized protein does not have antifungal activity. In our program, the tobacco mosaic viral vector system (Biosource) has been used successfully for the expression, in *Nicotiana benthamiana*, of osmotin (PR-5). The recombinant osmotin has the same antifungal activity spectrum of the native protein. A wheat PR-4 viral vector construct is being evaluated for recombinant protein production. In addition, a wheat PR-4 T-DNA binary vector has been constructed that can be used for transformation of peanut and other *Aspergillus*-affected crop species. Research on osmotin function indicates that growth inhibition of *Aspergillus nidulans* mediated by the PR protein is conditioned by heterotrimeric G-proteins that regulate the chitin composition in the wall. Based on analogy with the yeast model, osmotin activates a G-protein response in *A. nidulans* that facilitates its access to the plasma membrane, which is the site of antifungal function. Mutational analysis of osmotin indicates that specificity of recognition (toxicity) for target organisms resides on specific domains that may be subject to genetic engineering to tailor toxicity against specific microbes.

PURIFICATION OF TWO SMALL PEPTIDES WITH ANTIFUNGAL ACTIVITY AGAINST *ASPERGILLUS FLAVUS*

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In order to identify new genes encoding potent antifungal peptides, we have isolated a strain of *Bacillus subtilis* AU195 that inhibits *Aspergillus flavus* growth *in vitro*. The purpose of our study was to isolate the antagonistic substance produced by AU195 strain and to determine the chemical and antifungal properties of these molecules. The antifungal compounds produced by AU195 are two small peptides, very stable with a broad range of activity against fungi and a very high antifungal activity against *A. flavus*. We purified them with anion exchange and gel filtration column and determined with mass spectroscopy their molecular weight of 1045 and 1069 Da. Initial Edman sequencing was unsuccessful and tandem mass spectrometry of the molecular ion results in a highly complex spectrum which suggested a cyclic structure for both peptides. Both peptides have the same sequence after digestion at the Glu-C amino acid residue : STNYNPE. A modification of the Thr residue suggested the attachment of a hydroxy fatty acid of 15 carbons for the first lipopeptide and 16 carbons for the second lipopeptide. They belong to the iturin family and have the same amino acid sequence as bacillomycin D (Peypoux *et al.*, 1984).

Iturins are produced via the non-ribosomal biosynthetic pathway. Large multienzymes complexes, peptide synthetases, accomplished the synthesis of these lipopeptides. All peptide synthetase have a modular structure in which each module is responsible for one step in the assembly of the peptide antibiotic. The amino acids are activated in the form of aminoacyl adenylate and bound covalently via a thioesther linkage to the corresponding active site in the peptide synthetase. This is followed by peptide bound formation and transpeptidation of the growing peptide chain catalyzed by the cofactor 4'phosphopantetheine. Peptide synthetases consist of repetitive homologous domains of 600 amino acids. Each domain is responsible for the activation of one amino acid constituent of the peptide. Conserved core sequences can be identified among each domain and used to clone peptide synthetase. Two different approaches are currently investigated to isolate the peptide synthetase involved in bacillomycinD production. The first approach used the possibility of using degenerative primer to amplify and clone the conserved sequence of each domain. The second approach used Tn 917 transposon mutagenesis to create AU195 mutants unable to inhibit *A. flavus*. Tn 917 has already been used for identifying genes involved in surfactin (Nakano *et al.*, 1991) and fengycin (Chen *et al.*, 1995) production. To deliver Tn917 to the *B. subtilis* chromosome, temperature sensitive plasmids, such as pLTV1, derived from the *S. aureus* replicon pE194 have been constructed by Camilli *et al.*(1990). Protoplasts of AU195 have been transformed with pLTV1 and we are currently screening mutants which loose their antifungal activity against *A. flavus*. DNA fragments adjacent to Tn917 can be easily cloned into *E. coli* because the transposon derivatives carry ColE1 replication functions and antibiotic resistance genes selectable in *E. coli*. The transposon flanking DNA will be used to screen a l phage genomic library to isolate the peptide synthetase genes.

References

Camilli A., Portnoy D.A. and Youngman P., 1990, Journal of Bacteriology, 172: 3738-3744.

Chen C.L., Chang L.K., Chang Y.S., Liu S.T. and Tschen J.S.M., 1995, Mol. Gen. Genet., 248: 121-125.

Peypoux F., pommier M.T., Das B., Besson F., Delcambe L. and Michel G., 1984, J. Antibiot.; 37(12): 1600-4.

Nakano M.M., Magnuson R., Myers A., Curry J., Grossman A.D. and Zuber P., 1991, Journal of Bacteriology, 173: 1770-17.

GENETIC ENGINEERING AND BREEDING OF WALNUTS FOR CONTROL OF AFLATOXIN

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The tree nut crops in California, including walnuts, represent a 1.5 billion dollar industry, a significant amount of which is exported and thus threatened by the new regulations regarding aflatoxin contamination. Our approach to reducing aflatoxin has two components; 1) reducing the ability of tissues to support *A.flavus* growth and toxin production and 2) reducing infection opportunities through insect damaged tissues by developing insect-resistant plants.

Nuts of 26 walnut cultivars, representing a range of germplasm, were provided to the Western Regional Research Labs and evaluated for their ability to support growth of *Aspergillus flavus* and toxin production. Tulare walnut was found to be unique; it was both a poor host for *A. flavus* and appeared to inhibit aflatoxin production (Mahoney et al. unpublished). Additional Tulare nuts from a range of locations in California have been provided for additional assays. In addition, to determine whether the factors involved in this unique response are present in the seedcoat or cotyledons (nut meat), reciprocal crosses were made between Hartley (high aflatoxin producer) and Tulare. Since the seedcoat is maternal tissue and the cotyledons are derived from the cross-pollination, the response of the two populations of nuts will be similar if the cotyledon is involved and distinctly different if the seedcoat or maternal inheritance is involved. To further genetic analysis three seedling populations have been identified which represent hybrids between high x high, low x low and high x low toxin producers. Nuts of these populations will be available for assays.

The approach to reducing infection opportunities by enhancing resistance to insects has involved the expression of the *cry*IAc gene from *Bacillus thuringiensis*. We have previously demonstrated high levels of expression of a synthetically reconstructed version of *cry*IAc regulated by the CaMV35S promoter. This year we have continued to compare the expression of *cry*IAc under the transcriptional control of the CaMV35S and Ubi3 promoters using leaf tissue obtained from transgenic walnut plants introduced into the field. Direct feeding of embryos to first instar codling moth larvae revealed excellent mortality with both promoters. However, when larvae were fed leaf tissue expression was dependent on leaf age, particularly with the Ubi3 construct. Younger leaves showed expression but not older leaves. The CaMV35S promoter expressed well in both tissues and the levels in both exceeded Ubi expression. This year we completed the task of getting all of the transformed plants into the field at the two locations (Davis Pomology orchards and the USDA field facilities near Fresno). At this point we have 114 engineered plants in the field in Davis and 98 in Fresno not including controls.

In addition to *cry*IAc we have also been looking for other genes that have shown antifungal or anti-insect activity. In this context we have identified a full length polyphenol oxidase cDNA from walnut embryos. This cDNA was cloned into a binary vector containing a CaMV35S expression cassette. The resultant binary vector, capable of *in planta* expression of polyphenol oxidase and designated pDE98.0806, was introduced into *Agrobacterium* and used to transform tobacco. Fourteen transgenic tobacco plants were selected. Of these, 6 had high levels of GUS activity (a linked but unselected marker gene) and clearly discernable levels of polyphenol oxidase activity. Three plants that showed the highest levels of activity (10B, 10D and 12D) have been transferred to the greenhouse to be selfed to create homozygous lines. Antifungal and anti-insecticidal activity will be tested in the homozygous lines.

INHIBITION OF FUNGAL GROWTH BY PUTATIVE TRANSGENIC COTTON PLANTS

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We have regenerated more than two hundred cotton plants from several transformation experiments with the antifungal gene constructs encoding haloperoxidase or a synthetic peptide, D4E1. We previously reported that stably transformed tobacco plants expressing either haloperoxidase or D4E1 were obtained and significant antifungal activity in vitro against *Aspergillus flavus* was demonstrated using crude leaf extracts. In addition, the transformed tobacco plants were shown to inhibit the growth in vitro or in planta of other fungal plant pathogens such as *Colletotrichum destructivum*, *Verticillium dahliae*, *Fusarium moniliforme* and a bacterial phytopathogen, *Pseudomonas syringae* pv. *tabaci*.

Using crude leaf extracts from putative transgenic cotton plants we were unable to conduct antifungal assays similar to the tobacco model system due to the presence of phenolics and gossypol in cotton leaves which were themselves inhibitory to *Aspergillus flavus* growth. However, we were able to overcome this problem by using crude extracts from immature cottonseed (21 dpa) and conducted an initial screening of the putative R_0 transgenic cotton plants. Preliminary results indicate that cottonseed extract from some of the putative transgenic plants inhibit the growth of *Verticillium dahliae*. Immature cottonseed from these plants were also infected with a GFP-expressing *A. flavus* strain and the GFP fluorescence, as a function of the fungal growth and spread after one week of incubation, was evaluated under a GFP microscope and quantified using a fluorometer (HTS 7000, Perkin-Elmer). Some of the putative transgenic plants showed reduction in GFP fluorescence, thus indicating possible inhibition of *A. flavus* growth, as compared to the control seeds. Further characterization of the putative transgenic plants by molecular and additional antifungal assays are in progress.

ENGINEERING COTTON FOR RESISTANCE TO *ASPERGILLUS FLAVUS*: PROMOTERS AND STRUCTURAL GENES

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We are continuing our efforts to develop cotton that is resistant to the fungal pathogen, *Aspergillus flavus*. Our approach depends upon a consistent, efficient transformation/regeneration system, and the availability of suitable promoter/structural gene constructs to confer resistance. In the past, we optimized a transformation/regeneration system that relies on *Agrobacterium* mediated transformation of cotyledon tissue. Last year we experimented with biolistic transformation of suspension cell cultures, and embryogenic callus growing on solid media. Although we have had a difficult time maintaining sterile embryogenic cell suspension lines, we have been successfully maintained several independent embryogenic cotton lines on solid media. Our preliminary results from particle bombardment of this tissue indicate that we may be able to generate stable transformed embryos within 2-3 months after bombardment. This would result in a shorter time frame from transformation to generation of transformed plants than that required for the *Agrobacterium* mediated transformation/regeneration system.

To study structural genes that may confer resistance to *A. flavus* and other pathogens, we have created a series of transgenic *Arabidopsis* plants to study expression of these genes *in vivo*. Based on our challenges of these plants with a natural *Arabidopsis* pathogen, *Pseudomonas syringae* pv tomato, many of the structural genes studies were not effective in reducing growth. One of the most effective gene constructs against this pathogen was the haloperoxidase construct. This is one of three structural genes that we have already targeted for our cotton transformation experiments. In addition to the structural genes tested in the *Arabidopsis* challenge experiments (osmotin, lytic peptides, polygalacturonidase inhibitor, haloperoxidase, and basic bean chitinase/glucanase dual constructs), we have furthered our understanding of a set of naturally expressed cotton defense genes. This gene set encodes the Class I chitinases in cotton. We have identified three different chitinase genes (2 cDNA clones and one genomic clone). We have been able to partially purify a 30 kd protein with chitinolytic activity from induced cotton plantlets. Studies to finish the purification and enzymatic characterization of these proteins are in progress to compare the efficacy of the purified protein with that of other potential anti-flavus compounds.

The promoters that we have been studying include a cotton seed specific promoter (SSP), and a defense gene promoter. Tobacco seeds that express specific SSP/GUS reporter constructs have higher levels of GUS activity than seeds that express CaMV/GUS gene constructs. Deletion analysis of this promoter shows that it is possible to eliminate some of the tissue specificity, however the levels of expression are not comparable to those seen with the CaMV promoter. Our studies of the defense gene promoter are in the early stages. We are in the process of cloning various promoter deletion fragments into reporter vectors so we may track the levels of GUS in the fruit and seed after induction of expression.

TRANSFORMATION OF PEANUT WITH THE DEFENSIVE PEPTIDYL MIM¨ D5C

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The goal of this project is to transform peanut with a gene encoding an antifungal peptide, the membrane interactive molecule (MIM¨) D5C (Demegen Biotechnologies, Inc.). D5C is one of a class of synthetic peptides modeled after the structure of cecropins. The peptide, which forms an amphipathic (-helix, is thought to insert selectively into the fungal cell membrane to form pores which result in rapid ion movement producing osmotic shock. This peptide is highly active against A. flavus in vitro. Our working hypothesis is that accumulation od D5C in the intercellular spaces and/or walls of transgenic plant cells could retard the growth and reproduction of A. flavus and thereby reduce the severity of infection and the probability of aflatoxin contamination in the peanut. In the current study, 128 putativly transgenic lines were developed, representing16 transformaton events, i.e., multiple plants were developed from some events. Primary transformants were first analyzed by PCR to identify those plants which caried the D5C transgene and the hygromycin phosphotransferease (HPT) selectable marker gene. Because these gens were delivered in a single plasmid (i.e, marker and D5C were covalently linked) we anticipated that HPT (+), D5C (-) plants would be rare. However, hygromycin selection used in this set of experiments was relatively mild, so it seemed likely that some escapes would be observed.

Of 128 plants tested, 105 were negative for both HPT and D5C (HPT (-), D5C(-)). Eight lines carried only the HPT marker but were negative for the D5C gene. 15 lines were positive for both the D5C transgene and the HPT marker. Two untransformed lines wee retained for use as negative controls in further analysis, and the remainder of the untransformed lines wre discarded. Transgenic plants carrying both HPT and D5C were transferred to flats to permit pegging, and were transferred to the greehouse for subsequent growth.

D5C (+), HPT (+) plants were also analyzed by Southern blotting to determine the organizatd wth D5C did produce detectable amounts of the peptide.

R1 progeny were tested essentially as the R0 plants. A sample of three progeny were chosen randomly to represent each R0 line. All R0 plants that were positive for D5C by PCR produced PCR (+) R1 progeny. Southern blot analysis suggests that although there appear to be large blocks of linked transgene sequence, segregation among R1 plants does occur. Only one line (R0 progeny) failed to show evidence of segegation. This could represent sampling error or could indicate that this lineage is altered in some way.

Northern analysis of R1 progeny was generally uninformative, although there is some evidence of These plants were grown in a greenhouse maintained at approximately 30 C. Artificial lighting was used to extend daylength and augment ambient light in an attempt to increase pod set.

D5C showed strong activity against A. flavus in vitro. However, this observation left unanswered questions about the phytotoxicity of the constitutively expressed peptides, which might complicate recovery of transgenic plants. Toxic effects of the peptides could eliminate transgenic cells prior to plant regeneration, or could affect fertility of transformed plants. Further, it was not known whether observations in vitro would accurately predict efficacy against fungal growth in vivo, particularly if toxicity of the peptide effectively prevents recovery of plants expressing efficacious quantities of the compound.

A tobacco model system was used in parallel with peanut to study transformation with genes encoding D5C. Tobacco is easier and faster to transform than peanut, and transgenics can be obtained in relatively large numbers. Tobacco also has numerous fungal pathogens, facilitating test of antifungal activity. Two burley tobacco lines were transformed with D5C. Transformation was verified by Southern blots probed with the D5C, and Northern blot (mRNA) analysis. Preliminary Western blots are consistent with production of D5C. Detached leaves were inoculated with Rhizoctonia or Phytophthora.. Symptom development was delayed in transgenic leaves, and symptoms did not ever become as severe as those observed on controls.

Peanut cv. NC 7 was transformed with D5C using published protocols developed previously by Ozias-Akins and Weissinger laboratories. The transformation plasmid used in experiments reported here carry the coding sequence for D5C driven by the potato ubiquitin 7 (ubi 7) promoter. It also carries the selectable marker, hygromycin phosphotransferase, driven by a CaMV 35S promoter. The selectable marker and D5C genes are tightly linked.

Primary transformants (R0) were screened first by PCR to identify those plants which carried the gen of interest. 128 plants were tested using primer pairs that permit amplification of a sequence including all of the D5C coding sequence and its ubi 7 promoter. These plants wre independently tested for the presencce of PCR and Southern-blot analysis of the primary transformants and their self-progeny demonstrated the integration of the D5C transgene. Efficacy trials were carried out with clonally propagated R0 plants, using Cercospora arachidicola inoculation under greenhouse conditions as a model test system. Although symptom development was variable, several lines were identified which showed consistently enhanced resistance against Cercospora arachidicola than did controls. Importantly, there was complete correlation between the presence of the D5C transgene, as indicated by molecular analysis, and resistance against Cercospora.

R1 progeny of resistant primary transformants were also subjected to both molecular analysis and efficacy testing using the Cercospora arachidicola model system. The test was arranged in the greenhouse in a randomized complete block design, with 5 replicates, where replicates were rooted cuttings derived from a single plant. Test results were scored by counting lesion number at 2 and 4 weeks after inoculation. Lesion size and % damage, a subjective measure of disease development, were also recorded at 2 weeks, and % damage was recorded at 4 weeks post-inoculation. Of these measurements, only lesion number was sufficiently objective to permit showed apparent reduction of symptoms in some lines inoculated with the test fungus. It was not possible to demonstrate a statistically significant difference between transgenic progeny and control plants.

Subsequent tests of the progeny indicated that the transgene was not transcribed properly. It was determined that a likely cause for this loss of activity was homology-dependent silencing, possibly resulting from the use of two identical 35S promoters in the plasmid used to prepare transgenic peanuts. To correct this problem, a new plasmid vector was built which has an HPT selectable marker driven by a 35S promoter, while the D5C coding sequence is linked to a promoter (Ubi 7 Pro) from a ubiquitin gene derived from potato. This promoter, which shares very little homology with the 35S promoter has activity approximately equal to that of the 35S promoter, and also exhibits similar expression in various tissues.

A series of transformation experiments using this new plasmid (pRC9) and peanut NC7 resulted in the recovery of 62 plants representing 16 independent events. Of these, 20 carried only the HPT gene, while and additional 11 plants carried both the HPT and D5C genes. In an effort to advance potentially useful materials toward deployment as quickly as possible, no efficacy testing was carried out. Rather, the plants have been transferred to the greenhouse to make R1 seed, and samples have been taken for measurement of D5C peptide in the plants.

A major limitation has been the difficulty of detecting the D5C peptide in transgenic plants. Antiserum raised against D5C, originally provided under an agreement with Demegen, Inc., proved to be of insufficient titer to allow detection of the peptide at levels below 40ug in Western blot. D5C contains three thrombin cleavage sites which severely limit its antigenicity in rabbits.
In order to produce a more effective antiserum for peptide detection a synthetic peptide has been designed in which cleavage sites have been eliminated. The new peptide retains all of the carboxy-terminal of the original peptide, but eliminates the cleavage sites that had limited antigenicity of the molecule. This peptide design was provided to a commercial company, Quality Controlled Biochemicals, Inc., which synthesized the peptide. This truncated version of the D5C antigen was subsequently used to raise high-titer antiserum in rabbits.

Raw antiserum from these rabbits is now being used to develop more sensitive assays for expression of D5C in transgenic peanut tissue. Assay conditions were optimized to allow the detection of as little as 1.0 nanogram of the peptide by ELISA, an improvement of approximately 4 orders of magnitude over previous detection assays. This procedure made use of antiserum cross-absorbed against peanut protein extract in order to reduce non-specific binding. Additional antiserum will be purified on Protein A columns to remove constituents that might cross-react with proteins in peanut other than the target D5C. We anticipate that purification of the antibody may enhance sensitivity and should also improve its selectivity.

A preliminary test of 11 transgenic peanut lines from 3 independent events was inconclusive due to high background in non-transgenic controls. However, at least one of these samples repeatedly gave readings significantly higher than controls in replicated ELISA assays, suggesting that D5C may accumulate at detectable levels in at least some transgenics.

A great deal of additional work is required to demonstrate efficacy of antifungal peptides against A. flavus in peanut. Results reported here offer an important new tool which can be used to monitor expression of one such peptide. This in turn offers the opportunity to test the hypothesis that the presence of such toxic peptides will retard the growth and/or prevent the replication of invading Aspergillus species, thus contributing ion of introduced sequences. Of 15 lines tested, 14 appear to be derived from a single transformation event, the 15th producing a slightly different pattern on Southern analysis.

Northern blots were also performed on all transgenic lines to look for the presence of D5C mRNA. None of the lines tested produced the expected transcript of approximately 200 bp. Although this test was negative, it is still possible that the plants are transcribing only very small amounts of RNA, at levels below the detection limit of northern blotting. RTPCR is currntly being developed for use in this system in an attempt to detect very small amounts of the target RNA.
Finally, the R0 plants were tested for the presence of the D5C peptide using a Western blot procedure incorporating a new highly specific, high titer antiserum raised against a subunit of D5C. Detection limit of this test is nominally 1 ng of D5C in plant extract controls. None of the peanut plants were positive in this assay, but a tobacco line transforme to the elimination of aflatoxin in peanut and other crops.

GENETIC ENGINEERING OF PEANUT: PROGRESS WITH BT, PEROXIDASE, PEPTIDYL MIM D4E1, AND LIPOXYGENASE

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Our objectives for 1999 research included 1) completion of field testing of Bt (R_6) progeny for resistance to lesser cornstalk borer and reduction of aflatoxin contamination; 2) completion of molecular and efficacy analyses of transgenic plants containing the dual peroxidase/peptide construct; 3) begin to analyze expression of the soybean lipoxygenase gene in transgenic peanut plants.

Lesser cornstalk borer is known to inoculate peanut pods with the spores of fungi as the insect pest feeds on immature pods developing underground. By reducing the amount of scarification and penetration of peanut pods by the lesser cornstalk borer, we have hypothesized that we can reduce the pathways for entry of the aflatoxin-producing fungus, *Aspergillus flavus*, into the peanut seed. The route to insect resistance that we have chosen is the introduction of a synthetic *cryIA*(c) gene from *Bacillus thuringiensis* into the peanut cultivar, Marc-1. Lines with resistance to lesser cornstalk borer (LCB) were selected from the primary transgenics, and field tests for insect resistance were conducted in 1997, 1998, and 1999 with R2, R4, and R6 generations, respectively. Three lines which continue to show insect resistance and hygromycin resistance (22, 24, 137) have been selected as has one line that shows hygromycin resistance but does not contain a Bt toxin gene (124). According to Southern blot analysis, lines 22 and 24 likely originated from the same transformation event, but 137 was derived from an independent event. Although we anticipated that the 1999 field test with the R6 generation would include analyses for aflatoxin, the late planting date and persistent seed dormancy resulted in an insufficiently replicated test. Seeds will be harvested and used for an appropriately replicated field test in 2000.

The second of our three-tiered transgene approach to reducing aflatoxin contamination is to determine if overexpression of an anionic peroxidase gene and a peptide gene can singly or in combination reduce the growth or penetration of invading fungus. Transgenic plants overexpressing tomato anionic peroxidase (tap1) have been recovered and analyzed at the RNA and protein levels. Two plants showing tap1 transcript accumulation also show the tomato isozyme form on isoelectric focusing gels. Another plant with a transcript of the expected size as well as a smaller transcript hybridizing with tap1 shows an overall reduction in peroxidase activity, both anionic and cationic. Preliminary analysis of potential insect resistance in peroxidase overexpressors suggests an increase in resistance. The Demegen peptide gene for D4E1 has been introduced into peanut as a ubiquitin translational fusion under the control of the potato ubiquitin 3 promoter. Although transcript levels are high in some lines, due to the lack of an antibody to D4E1, we have been unable to determine if the free peptide is produced by cleavage from the ubiquitin protein. Our results with in vitro bioassays using peanut leaf extract and free peptide suggest that the peptide is rapidly degraded by the extract. These results with leaf extract are consistent with two pathogens, *Aspergillus flavus* which is moderately inhibited by 10 μM peptide in PBS or water, and the bacterium, *Acidovorax avenae* which is strongly inhibited at the same concentration.

For reduction of aflatoxin biosynthesis, introduction of the soybean lipoxygenase gene, lox1 (driven by the carrot DC3 promoter) from Nancy Keller has been accomplished and plants containing the gene have been regenerated. The DC3 promoter is a seed specific promoter that also should be inducible by abscisic acid. Preliminary analyses for lox1 expression in transgenic R1 progeny are inconclusive. In one line from which progeny tested positive for the presence of the gene, no expression was observed in leaves treated with ABA or left untreated. This result could be interpreted as a case of gene silencing or lack of promoter response to ABA. Expression in seeds will be determined as soon as material is available.

POSTER PRESENTATIONS

PLANT REGENERATION FROM TRANSGENIC PEANUT LINES (*ARACHIS HYPOGAEA* L.) TRANSFORMED WITH A TOMATO ANIONIC PEROXIDASE GENE

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Aflatoxin, a carcinogenic mycotoxin, is produced by *Aspergillus flavus* and *A. parasiticus* after the fungi enter pods through cracks caused by environmental stress or insect damage. Conventional breeding is being used to enhance fungal resistance, but other strategies, such as genetic engineering, are being pursued as. Transgenic antifungal technology is an adjunct for reducing aflatoxin contamination of peanuts. In order to test the efficacy of reported antifungal genes, we have introduced a tomato anionic peroxidase (*tap*1 from P. E. Kollatukudy, Ohio State University) under the control of the CaMV35S promoter into embryogenic cultures of three peanut cultivars, i.e., Florunner, Georgia Runner, and Georgia Green via particle bombardment. The integration of peroxidase and marker genes in hygromycin-resistant regenerants was confirmed by the polymerase chain reaction and Southern hybridization analysis. Ninety-seven percent of the hygromycin-resistant plants showed amplification of a 468 bp fragment from the coding region of *tap*1, and the 3 kb *tap*1 gene was detected by Southern hybridization in 96% of the plants tested. Plants transferred to the greenhouse have flowered and set seeds. Molecular analysis for gene expression and bioassays for fungal resistance are in progress.

TRANSFORMATION OF PEANUT WITH A SYNTHETIC ANTIFUNGAL PEPTIDE GENE

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Pre-harvest contamination of peanut (*Arachis hypogaea* L.) with aflatoxin, produced by *Aspergillus flavus* Link, could be reduced by protecting immature pods from fungal invasion and/or growth. Our lab is genetically engineering peanut with antifungal genes in an attempt to enhance plant resistance to fungal diseases. A synthetic antifungal peptide gene was introduced into peanut via microprojectile bombardment of somatic embryogenic cultures. Expression of the peptide gene is regulated by the ubiquitin promoter from potato fused with the potato ubiquitin 3 coding sequence. Constructs also contained the *hph* gene for resistance to the antibiotic hygromycin as a selectable marker. Forty-five hygromycin resistant embryogenic cell lines were obtained in 12 experiments. Transgenic tissues from almost all cell lines were easily regenerated into whole plants. Southern blot analysis demonstrated stable integration of the peptide gene into the peanut genome and revealed that the transgene was inserted in different loci and copy numbers among the independent transformation events. The peptide gene expression was detected in the form of fusion transcripts with the ubiquitin coding sequence. A transcript for peanut ubiquitin also could be detected which had a larger size in comparison to the potato ubiquitin-peptide fusion transcript. Bioassays for antifungal activity are in progress.

SEQUENCE ANALYSIS OF A CORN CHITINASE FROM TEX 6

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Tex-6 corn is an inbred line that has been shown to be resistant to Aspergillus flavus pathogenesis. Our lab has developed an assay which is capable of quantitatively measuring fungal growth inhibition. Inhibitory activity in Tex-6 has been associated with two bands which repeatedly appear on SDS-PAGE. These two bands have been shown to be chitinases. Our data suggests that the majority of the antifungal activity in Tex-6 corn is associated with these two proteins. We havepurified the chitinase that we believe to be the more active of the two and have sequenced portions of this protein. We are now using the amino acid sequence data to find the gene for this protein. It is our hope that there is a novel quality in the Tex-6 chitinase/s which makes these enzymes more robust than other plant chitinases characterized to this point.

MAIZE TRANSFORMATION FOR RESISTANCE TO ASPERGILLUS EAR ROT AND AFLATOXIN PRODUCTION

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Chitinase and β-1,3-glucanase are known antifungal proteins that have been implemented as being at least part of the mechanism of resistance to Aspergillus ear rot and aflatoxin production. The purpose of this research is to transform corn plants with genes that produce these two proteins and to determine their usefulness in control of Aspergillus ear rot and aflatoxin production.

For transformation, embryogenic calli induced from immature embryos of H99 and Pa91 were used for particle bombardment. Constructs used for transformation were pGlbGlu II containing β-1,3-glucanase gene from maize and pGlbCh18 containing chitinase 18 from *Phaseolus vulgaris*. Both genes were driven by globulin 1 promoter from maize. The *bar* gene was used for selection marker in both constructs.

Sixteen different transformation events have been obtained and self-pollinated up to six times. Crosses also have been made with B73 and Mo17 and self and backcross generations have been produced for further study. The transgene expression has been detected in embryos and determined to be transmitted in pollen. The globulin 1 promoter from maize has been reported to be embryo-specific, however, we also found transgenic expression in endosperm and pericarp. With specific transformations the activity of chitinase and β-1,3-glucanase were higher in embryos, endosperm, and pericarp and aleurone of transgenic lines than those of H99xPa91 but not higher than in the resistant inbred Tex6. The kernel screening laboratory assay indicated that one transgenic line with the β-1.3-glucanase gene and one with the chitinase gene were more resistant to *A. flavus* than H99. GUS activity of the H99 kernels was 7.0 and 2.2 times higher than that of the two transformations. The transformations, however, were very similar in GUS activity to resistant inbred Tex6.

Last summer we inoculated a large number of transgenic plants for disease resistance in the field and preliminary results have shown that they are resistant. We will be further evaluating these lines and should be able to report the results next year.

CHARACTERIZATION OF AN ALKALINE PROTEASE EXCRETED BY *ASPERGILLUS FLAVUS* IN INFECTED CORN KERNELS

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A 33 kDa protein predominantly present in *A. flavus* infected corn embryo tissues was identified as an alkaline protease based on N-terminal amino acid sequence homology. The corresponding genomic DNA was cloned and sequenced. It shares over 99% homology with the genomic DNA sequence of alkaline protease from *A. oryzae*, and 80% homology with elastase from *A. flavus*. In 10% PDB medium, the expression of this protein increases with culture time and peaked at 3 days (40%). However, the level of this protein was much lower when *A. flavus* was grown in A&M medium supplemented with 0.5% gelatin, and it was not expressed in A&M medium supplemented with 0.5% starch. Using Mono-S column this protein was purified in one step from concentrated culture filtrates. In gelatin cup-plate assays, the activity of purified enzyme was inhibited over 92.8% in the presence of 0.5 mM PMSF, and 23.8% in the presence of 200 µg/ml of the 14 kDa corn trypsin inhibitor. However, the activity of this enzyme was not affected by the presence of 200 µg/ml of trypsin inhibitor from soybean or chymotrypsin inhibitor from potato. The presence of protease inhibitors on kernel surfaces also affected aflatoxin production in *A. flavus* infected corn kernels. Kernels precoated with 5 mM PMSF supported 6 to 14 fold more aflatoxin B1 than non-treated control. However, Kernels precoated with other protease inhibitors supported similar levels of aflatoxin production compared to the control.

These results suggest that the main function of this alkaline protease may be to degrade proteins and to provide the fungus with nutrients. Meanwhile, degradation of kernel embryo proteins may also effectively prevent the host from establishing an active (inducible) defense system during infection to help the colonization of corn kernels. The data also suggest that inhibition of fungal proteases may force *A. flavus* to use carbohydrates as carbon source which upregulates genes involved in both sugar utilization and aflatoxin biosynthesis, therefore lead to increased aflatoxin production when it is inhibited by PMSF. Several studies have found that in *A. flavus* genes involved in sugar utilization are not only physically located in the same cluster as, but also co-regulated with genes involved in aflatoxin biosynthesis (Yu et al., 1999).

CHARACTERIZATION OF *CSSAP92*, A CORN GENE RESPONSIVE TO *ASPERGILLUS FLAVUS* INFECTION

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Corn kernels are highly susceptible to *Aspergillus* infections and aflatoxin contamination. The lipoxygenase (lox) enzymes are thought to play a role in the *Aspergillus*/plant interaction, and this project involved identifying and characterising *lox* genes that were expressed in the corn embryo. Screening 11 non-homologous cDNA clones for embryo-specific expression, we identified one *lox* clone, CSSAP 92, that was clearly expressed in germinating and control embryos, but repressed in *A. flavus* infected embryos. Moreover, this putative *lox* gene was expressed differently in different corn lines. CSSAP92 was sequenced and found to encode a full length transcript for the designated *lox* gene *cssap92*. *Cssap92* is 96% identical to a previously described *lox* from corn, *lox2*, at the nucleotide level and is likely to be an allele of that gene. Activity shows that it preferentially adds oxygen to the 13th carbon of its linoleic acid substrate.

USE OF A GREEN FLUORESCENT PROTEIN EXPRESSING *ASPERGILLUS FLAVUS* STRAIN TO ASSAY FOR ANTIFUNGAL FACTORS IN COTTONSEED

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An *Aspergillus flavus* strain was engineered to express the green fluorescent protein (GFP) from the jellyfish *Aequora victoria*. The bright green fluorescence of the *A. flavus* strain expressing GFP (*A. flavus* strain 70-GFP) made it possible to visualize the inhibitory effect of a purified antifungal peptide (D4E1) on conidial germination and subsequent fungal growth. Using *A. flavus* strain 70-GFP we were able to identify and monitor specific cotton tissues colonized by this fungus in greenhouse grown plants. A detached seed colonization assay was developed to screen immature seeds from putative transgenic cotton plants engineered to express antifungal peptides and proteins to inhibit colonization by *A. flavus* strain 70-GFP. Differences in fungal colonization of cottonseeds were measured using both GFP-fluorescent microscopy techniques and quantitative fluorometry using an HTS 7000 (Perkin-Elmer) fluorometer.

This work demonstrated the utility of an *A. flavus* strain expressing GFP. GFP expression did not interfere with pathogen aggressiveness as determined in boll inoculation experiments. Boll inoculation experiments also indicated that fluorescence due to GFP could be distinguished from fluorescence due to BGYF using the correct GFP filter combination in the HTS 7000 fluorometer. The detached seed colonization assay indicated that *A. flavus* strains expressing GFP should be very useful for rapidly identifying cotton lines with enhanced resistance to *A. flavus* colonization developed through genetic engineering or traditional plant breeding. The ease with which tissues colonized by GFP expressing *A. flavus* strains can be identified should also make GFP an attractive tool for studying the ecology, etiology, and epidemiology of cotton bollrot caused by *A. flavus*.

DROUGHT INDUCED TRANSCRIPTIONAL CHANGES IN PEANUT (*ARACHIS HYPOGAEA* L.)

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Pre-harvest contamination of peanut (*Arachis hypogaea* L.) with aflatoxin, a carcinogenic fungal secondary metabolite, is a recurrent problem especially under drought and elevated soil temperature conditions. To understand the molecular mechanism and differential gene expression under irrigated and water stress conditions, 45 days old seedlings of peanut cultivar Florunner were subjected to water stress for 15 days. The mRNA from the leaf tissue of stressed and non-stressed plants were isolated and cDNA molecules were synthesized in vitro. These molecules were amplified following Differential Display Reverse Transcribed - Polymerase Chain Reaction (DDRT - PCR). The DDRT products from stress and non-stress samples were resolved on a sequencing gel to compare qualitative and quantitative differences in the gene expression. A total of 24 primer combinations were tested. We have identified a total of 43 mRNAL transcripts, which are affected due to water stress. Most of the transcripts showed quantitative effect leading to overexpression or suppression of genes following water stress. In addition, transcripts that are turned-on or turned-off in response to water stress have also been identified. These results showed importance of using molecular approach that would be helpful in understanding molecular events associated with resistance to fungal invasion, aflatoxin contamination and water stress in peanut.

MICROBIAL ECOLOGY

PANEL DISCUSSION

PANEL DISCUSSION TITLE: MICROBIAL ECOLOGY

PANEL MEMBERS: Gary Windham (Chair), Larry Antilla, Peter Cotty, Joe Dorner, Bruce Horn, Sylvia Hua, Jay Mellon, and Merritt Nelson

SUMMARY OF PRESENTATIONS: Sylvia Hua gave an update on her work using saprohytic yeasts to reduce aflatoxin contamination in almonds and pistachios. Petter Cotty and Joe Dorner presented results on using biological control to reduce aflatoxin levels in cotton and peanut. Larry Antilla described the progress made this year in producing large quantities of an atoxigenic strain of *A. flavus* for application in selected Arizona cotton fields. Jay Mellon presented data on the sequence of substrate utilization during corn kernel infection and subsequent aflatoxin contamination. Merritt Nelson described the use of sentinel sites for monitoring *A. flavus* community structures in desert agricultural soils in Arizona. Bruce Horn described the role of vegetative compatibility in the biological control of aflatoxingenic fungi. Gary Windham presented information on the localization of *A. flavus* and aflatoxin in developing ears of corn using *A. flavus* transformants containing GUS reporter genes.

SUMMARY OF PANEL DISCUSSION: Gary Windham was asked about *A. flavus* spread in cobs of developing ears. Windham stated that the fungus moved readily in the vascular tissue of cobs, and kernels associated with the infected vascular tissue were usually also infected by the fungus. Dave Wilson asked Joe Dorner and Peter Cotty what percentage of control would be considered successful in controlling aflatoxin in peanuts and cotton using biological control. Much discussion followed on what percentage of control was needed to be considered "successful". Don Wicklow asked Joe Dorner about the capability of Koji molds to be human pathogens. Dorner reported that he was not aware of any reports of Koji molds being human pathogens and that these molds are commonly used in Japan in fermentation processes. However, fungi not usually considered human pathogens may present problems as an allergen and for anyone with a suppressed immune system. Hamed Abbas asked Peter Cotty to describe aerial application of atoxigenic strains in Arizona. Bruce Horn was asked several questions concerning the role of vegetative compatibility in the biological control of aflatoxigenic fungi. Ed King asked the panel what was the lowest possible cost for applying an effective dose of material containing atoxigenic fungi in regards to controlling aflatoxin contamination. At present, costs are approximately $10.00/acre for application of 10 lbs material/acre. Larry Antilla said they would like to apply more material per acre and hopefully keep costs at $20.00/acre or less. As the system for producing inoculum improves and as volume increases, the cost for applying atoxigenic fungi is expected to be even less. A discussion followed whether "more" was better in regard to the optimal amount of atoxigenic fungi to use in field applications. Also, the use of wheat as the carrier for atoxigenic fungi was discussed. Ed King asked would it be economically feasible to use the Arizona system of biocontrol in corn in the Mid-South. Peter Cotty said corn producers did not think it would economically feasible in the South. Jane Robens asked where could the Arizona system be tested on corn. Cotty said that south Texas and Arizona would be likely areas to test the system on corn. Joe Dorner said they had tried biological control of aflatoxin on corn in south Georgia. They saw reductions of aflatoxin by 66-88% in corn. Dorner was hesitant to suggest the biological control system would be economical with the current price of corn. Cotty said for corn producers to benefit, corn fields in a large area would need to treated. Participation by all growers in a large area is critical for success. Dave Wilson asked Cotty and Dorner if they had ever applied toxigenic strains following application of atoxigenic strains. Natural populations were considered to be at a high enough level that application of toxigenic strains was not necessary. Phil Wakelyn and Themis Michailides questioned Cotty on sampling procedures to determine the number of air-borne spores. Samples were collected weekly

and then a dilution plate method was used to quantify the number of air-borne spores. Nancy Keller asked Sylvia Hua when saprophytic yeasts would be available to release in field studies. Jane Robens asked Cotty if it was important to use native atoxigenic fungi when making field applications. To have the most residual activity and most stable activity, it is very important to select isolates from your region particularly if soil types are different. Isolates adapted to the acidic soils found in the Southeast may not work in other areas of the country. Specific isolates might be needed to treat fields in Georgia, Mississippi, Texas, etc. Mixtures of isolates might be used treat fields to have fungi fill a specific niche. A discussion was initiated by Jane Robens on the response of EPA to treating fields with a number of isolates. EPA presents a number of hurdles before field applications can be made on a large scale. The use of native atoxigenic strains would be viewed favorably by EPA. Hamed Abbas asked the panel what would be the effect of aflatoxin on plant growth. Would the plant degrade aflatoxin? These type studies have been reported in the literature. Neil Widstrom reported that Marcus Zuber and Ivan Lillehoj had conducted these type studies.

PLATFORM PRESENTATIONS

BIOCONTROL RESEARCH TO REDUCE AFLATOXIN IN ALMONDS AND PISTACHIOS

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While commercial production of almond in California has been for about 100 years, and over 50% of the production is exported, pistachio is a recently introduced new crop in this state. The annual market value for these two crops is approaches 1.2 billion dollars. Both domestic and export markets of these nuts presently allow a maximum level of aflatoxin B_1 contamination in the edible nuts to be 20 ppb. Even very low degrees of infection of the nuts by *A. flavus* can result in aflatoxin levels above these mandatory standards.

The unique nature of aflatoxin problem in food and feed stuffs is due to several factors: *A. flavus*, adapts saprophytically on organic debris in the field and reproduces copiously; has a broad ecological niche; produces aflatoxin in almonds and pistachios; can not be controlled by conventional technologies used for plant pathogens. During the month of August and September *Aspergillus flavus* were found in the collected air samples from orchard indicating that there is a build up of this fungus in the environment. Large acreage of almonds and pistachios promotes the accumulation of a large population of this fungus. Biological control to reduce the number of *A. flavus* in orchards may be helpful to decrease infection and thus aflatoxin content in edible nuts.

Saprophytic yeasts, which can colonize plant surfaces for very long periods of time under dry conditions, produce extracellular polysaccharides that enhance their survivability and restrict both colonization sites for and nutrient flow to fungi. Some of the yeast species have been developed to control post harvest fungal disease of fruits. In the past few years over two hundred yeasts have been isolated in our lab. Thirty strains have been identified belonging to twenty different species. A large number of yeasts were tested for their antagonistic effect on *A. flavus* by a visual bioassay developed in WRRC. The yeasts which were shown to inhibit aflatoxin accumulation were further screened for their biocontrol efficacy on plant tissues such as almond leaves, fruits and nuts. The number of yeasts and fungal spores of *A. flavus* were enumerated by determining the CFU (colony forming unit). The population of yeasts and fungal spores can be further analyzed in a Beckman Coulter Multisizer counter. A Biolog system are being used to analyze the nutritional utilization of the yeasts. The results from the Biolog system may provide useful information to prepare inocula for field application.

Since field tests are complex and laborious, cooperative association between the public and commercial sectors is essential for successfully introducing " **saprophytic yeasts**" the biocontrol agents to the orchards.

LONG-TERM INFLUENCES OF ATOXIGENIC STRAIN APPLICATIONS ON *ASPERGILLUS FLAVUS* COMMUNITIES IN COMMERCIAL AGRICULTURE

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Aspergillus flavus, the causal agent of aflatoxin contamination of cottonseed, is a complex species composed of many distinct vegetative compatibility groups (VCGs). Isolates belonging to different VCGs may produce widely different quantities of aflatoxins. Some naturally occurring isolates of *A. flavus* produce no aflatoxins. Some of these atoxigenic strains have the ability to competitively exclude aflatoxin-producing strains during crop infection and thereby reduce aflatoxin contamination. In both greenhouse and field-plot tests atoxigenic strain efficacy has repeatedly been demonstrated. *A. flavus* communities resident in soils vary among agricultural fields in aflatoxin producing capacity. Field-plot tests suggested that applications of atoxigenic strains may provide long-term reductions in the aflatoxin producing potential of fungi resident in treated fields. Tests to evaluate the longevity of changes to *A. flavus* communities induced by atoxigenic strain applications were initiated in 1996 under an Experimental Use Permit (EUP) issued by the EPA. The experimental program outlined in the EUP called for treatments over a three year period (1120 acres total) and for monitoring the *A. flavus* community from 1996 through 1999. Different treatment regimes were applied to different fields with some fields receiving treatment only in a single year and others receiving treatments in multiple years.

Sterile wheat seed colonized by an atoxigenic strain was applied to 22 fields ranging in size from 10 to 160 acres from 1996 to 1998. The material was applied either by air or ground at the rate of 10 lb per acre. In order to monitor changes to the composition of *A. flavus* communities, soil samples were collected prior to application each year. From 1996 through 1999 over 8,000 isolates of *A. flavus* were cultivated from soil samples taken from the treatment areas. Isolates were characterized by strain and those assigned to the L strain of *A. flavus* were further characterized by vegetative compatibility analysis in order to determine applied strain distribution.

One year after application, atoxigenic strain incidence was greatly increased and incidence of the highly toxigenic S strain was greatly decreased in treated and adjacent fields. The applied strain incidence gradually declined by the second year after application. However, even with this decline, the atoxigenic strain remained in treated fields at levels significantly higher than prior to treatment. The incidence of the applied strain in fields adjacent to treated fields was variable indicating possible directional movement of the strain from treated to untreated fields. In some locations crop and crop stage were apparently important determinants of influences beyond treated fields.

One of three fields treated in 1996 was not subsequently treated. Incidence of the atoxigenic strain went from 1.8% prior to treatment to 96% one year after, 52% two years after, and 47% three years after treatment. Long-term influences on the incidence of the S strain also occurred with a 52% incidence pretreatment and only a 2% incidence three years after application. Overall, the results suggest that long-term useful reductions in the aflatoxin-producing potential of fungal communities can be achieved by atoxigenic strain application.

PRODUCTION OF COMMERCIALLY USEFUL QUANTITIES OF ATOXIGENIC STRAIN INOCULUM

Larry Antilla[1] and Peter J. Cotty[2]; [1]Arizona Cotton Research and Protection Council, Tempe, AZ, [2]Southern Regional Research Center, USDA, ARS, New Orleans, LA

The Arizona cotton industry is facing a serious economic crisis. Declining prices, increasing production costs as well as a significant revenue loss due to high aflatoxin levels are all major factors contributing to the current deterioration of the cotton production system. In Arizona, direct losses of $20 - $40 per ton frequently result from cottonseed having an aflatoxin content over 20 ppb. High aflatoxin levels may also prevent access to markets outside of the state. Thus, loss of Arizona's last cottonseed oil mill leaves many growers with no outlet for contaminated seed.

Successful multi-year testing of atoxigenic strain technology developed by the Agricultural Research Service of the USDA led the Arizona Cotton Growers Association leadership to make a bold move in the spring of 1998. The Arizona Cotton Research and Protection Council (ACRPC) was directed to develop and make functional a grower owned facility for the production of commercially useful quantities of atoxigenic strain inoculum and to develop an area-wide aflatoxin management program utilizing this new technology. This report outlines progress that has been made toward the achievement of that goal.

PESTICIDE REGISTRATION: In June 1998 the ACRPC submitted an application to EPA for a Section 3 registration for use of *Aspergillus flavus* AF36 on cotton in Arizona. Included in the request was an option for expanding the existing Experimental Use Permit (EUP) to allow treatment of up to 20,000 acres of cotton in Arizona with AF36. On May 26, 1999 EPA granted the expanded EUP as a means to develop critical data thought necessary for full registration.

AF36 PRODUCTION FACILITY DEVELOPMENT: In September, 1998 the ACRPC leased a 15,000 square foot warehouse for development of AF36 manufacturing. Included in the site plans were laboratories for commercial scale inoculum production and assessment of field program results. Due to extreme time constraints, construction of the facility was carried out in two incremental phases: Phase I -- Manufacturing. Infrastructure for AF36 production including a processor (Littleford 2000 liter blender), support equipment and utilities began in mid-January and was completed by the end of March, 1999. Phase II -- Labs and Offices. Three labs; two for the production and maintenance of pure AF36 inoculum and a third for assessment of area-wide field results were constructed between mid-April and June, 1999.

ATOXIGENIC STRAIN PRODUCTION: Techniques for the production of sufficient quantities of AF36 inoculum for commercial scale production were developed at the Southern Regional Research Center in New Orleans. ACRPC personnel were trained in these techniques which were then successfully utilized to produce the inoculum required by the Phoenix facility during 1999.

1999 PROGRAM ACTIVITIES: Construction delays and difficulties in obtaining replacement parts needed to refurbish the used Littleford blender prevented achievement of the initial goal of treating 20,000 acres of cotton in up to 6 test areas in Arizona. However, three treatment areas were established and a total of 10,388 acres received AF36 applications during the 1999 crop year. In each area treated and not-treated areas were established with at least a 1-mile buffer between. Evaluation fields (55 total) were established within both treated and not-treated areas. Both crops and soils from evaluation fields are being monitored in order to assess program efficacy and to provide a data base with which to optimize further implementation of atoxigenic strain technology. Additionally, a total of 6 Burkard cyclone air samplers were placed in treated and not-treated areas.

Air collection samples from the cyclones are being obtained weekly and submitted to the ACRPC assessment lab for quantitation of *A. flavus* inocula. This will provide additional information on the influence of applications on the quantity of *A. flavus* in the air. The treated test areas were as follows: Roll, AZ (Yuma County) -- 1,241 acres treated: Paloma, AZ (Maricopa County) -- 6,426 acres treated: Maricopa, AZ (Pinal County) -- 2,721 acres treated.

PROGRAM EVALUATION: Soil Sampling – Pre-treatment soil samples were taken from all evaluation fields to establish baseline levels of *Aspergillus flavus* strains. All fields will be resampled prior to program initiation in the year 2000. Toxin Levels -- Cotton modules on all evaluation fields are being tagged for separate ginning and evaluation of toxin levels in seed in both treated and untreated control areas. Gins have been asked to monitor the distribution of contamination across treated and untreated areas.

PROGRAM COSTS: To date, the total investment of Arizona cotton grower's in this program is estimated to be approximately $1,061,000.

ISSUES TO BE ADDRESSED:

1. Expanding and improving manufacturing systems.
2. Supplying data required by EPA for Section 3 registration.
3. Improving AF36 field delivery systems. 4. Identifying cultural practices that optimize strain success.

POTENTIAL OF THE KOJI MOLDS, *ASPERGILLUS ORYZAE* AND *ASPERGILLUS SOJAE*, AS COMPETITIVE FUNGI IN BIOLOGICAL CONTROL OF AFLATOXIN CONTAMINATION

Joe W. Dorner, Richard J. Cole, and Bruce W. Horn: USDA, ARS, National Peanut Research Laboratory, Dawson, GA

Aspergillus oryzae and *A. sojae* are often referred to as koji molds because they are used in fermentations to produce koji, a fermented soybean and wheat mixture, which is used to produce products such as soy sauce, sake, and miso. These fungi have been used safely in oriental fermentations for centuries. *A. oryzae* and *A. sojae* are closely related to the aflatoxin-producing fungi, *A. flavus* and *A. parasiticus*, respectively, and are considered domesticated strains of these fungi. *A. oryzae* and *A. sojae* have never been shown to produce the aflatoxins and they are also nonpathogenic to humans. These fungi were tested during 1997 and 1998 as potential biocompetitive fungi against preharvest aflatoxin contamination of peanuts.

In 1997, a heavy inoculum (100 pounds per acre of fungal infested rice) of each of four strains of *A. oryzae* and five strains of *A. sojae*, obtained from the ARS culture collection in Peoria, IL, were applied to two replicate peanut plots approximately 50 days after planting. Peanuts were subjected to late-season drought conditions which have been shown to be optimal for preharvest aflatoxin contamination. Soil microflora at harvest was dominated by the applied strains of *A. sojae*, and average aflatoxin values for all peanuts was reduced by 84.1% compared with untreated controls. Average aflatoxin values in edible peanuts were reduced by 98.5%.

In 1998, one strain each of *A. oryzae* and *A. sojae* that were most dominant in 1997 were selected for further study. Also included was a strain of each species obtained from the Japanese fermentation industry. The ARS and Japanese strains were used to inoculate 8 replicate test plots, and peanuts were again subjected to late-season drought conditions. Aflatoxin contamination in all shelled categories of peanuts from plots treated with strains from the ARS culture collection was reduced by 69.7%, although this reduction was not statistically significant. The reduction in aflatoxin in edible category peanuts was 77.2%, and this was statistically significant ($P < 0.05$). Peanuts from plots treated with the koji molds obtained from Japan showed reductions of 9.6% for all peanuts and 10.9% for edible peanuts, but neither of these reductions was significant.

These results show that there may be some promise for use of certain strains of *A. oryzae* and *A. sojae* in the biological control of aflatoxin contamination of peanuts. However, they probably do not warrant a changeover in the makeup of biological control formulations from non-toxigenic strains of *A. flavus* and *A. parasiticus* to *A. oryzae* and *A. sojae* unless difficulties are encountered in the registration of a biopesticide that includes *A. flavus* and/or *A. parasiticus*.

SEQUENCE OF SUBSTRATE UTILIZATION DURING CORN KERNEL INFECTION AND AFLATOXIN CONTAMINATION

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The commercial value of corn is diminished by contamination with aflatoxins, potent carcinogens produced by the fungus *Aspergillus flavus*. Carbohydrates, storage proteins and lipids of corn seed represent nutrient sources that this fungus can potentially convert to aflatoxin. A defined fungal growth medium containing the major corn reserve materials starch, triglycerides (corn oil), and zein (corn storage protein) in proportions approximating those in mature seed was used to assess certain fungal parameters in a timed study (7-day). The fungus initiated hydrolysis of both starch and triglycerides within 24 h. Peak concentrations for glucose and its oligomers (dimer, trimer, etc.) occurred at day 2. These saccharide substrates were consumed throughout the fermentation period. Peak concentrations for linoleic, oleic, palmitic and stearic acids also occurred at day 2, followed by rapid decreases. Aflatoxin concentrations rose rapidly after day 1 with a peak at day 4 and paralleled biomass production. Medium pH began to decrease as aflatoxin production began, as the two parameters appear to be interrelated. Zein concentrations decreased gradually throughout the fermentation period. Mannitol was the fungal metabolite produced in highest concentrations, with a peak at day 4. Other fungal metabolites detected included kojic acid, arabitol, erythritol and trehalose. Both carbohydrate and lipid substrates appeared to drive aflatoxin and biomass production in the simulated corn medium.

A timed study was also carried out with *A. flavus*-inoculated whole corn kernels to monitor these fungal parameters. Whole corn seed was surface-sterilized, inoculated with *A. flavus* (25% moisture), and incubated at 31°C for up to 12 days. After the appropriate incubation period, corn samples were dried at 55°C for 24 h and ground into meal for further analysis. Aflatoxin concentrations were determined by thin layer chromatography. Ground corn samples were extracted with water for saccharide analysis and methanol/chloroform for lipid analysis. Extracts were derivatized by silylation and analyzed by gas chromatography. Sucrose concentrations dropped rapidly after day 2 in the inoculated corn, but not in the noninoculated corn. Likewise, raffinose concentrations rapidly decreased after day 4 in the inoculated corn. Glucose levels remained similar in both inoculated and noninoculated corn until after day 6. Triglyceride concentrations began to significantly decrease in the inoculated seed after day 2. Aflatoxin concentrations increased sharply after day 2 until day 12. Erythritol was the fungal metabolite produced in the highest concentrations with a peak (3.5 mg/g) at day 8. This metabolite was produced at much lower concentrations in the liquid medium. Arabitol and mannitol were both produced at concentrations about one half those of erythritol. *Aspergillus flavus* preferentially utilized saccharide substrates in whole corn before hydrolyzing triglycerides and starch. Saccharides may be required for rapid induction of aflatoxin biosynthesis in corn. Sugar alcohols are major metabolites of *A. flavus* during utilization of corn substrate.

SENTINEL SITES FOR MONITORING *ASPERGILLUS FLAVUS* COMMUNITY STRUCTURE IN SOUTHWESTERN DESERT AGRICULTURAL SOILS

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Monitoring the composition of Aspergillus *flavus* communities is an important part of an aflatoxin management program based on biological control using naturally occurring atoxigenic strains of the fungus. We have studied the spatial characteristics of *A. flavus* S strain incidence over the past five years using geographic information systems and geostatistics. These studies have allowed us to document both consistency and spatial variability in *A. flavus* community structure and to develop predictive maps to monitor trends in *A. flavus* strain composition in Yuma County. In 1998, we began to apply these techniques in cotton-growing areas statewide. The primary goal of our work in 1999 is to develop baseline data on the incidence of the atoxigenic *A. flavus* AF36 and the highly toxigenic A. *flavus* S strain prior to large-scale treatment of cotton fields using AF36. This is a collaborative study among the University of Arizona Department of Plant Pathology, the Arizona Cotton Research and Protection Council (ACRPC), and the Southern Regional Research Center of the ARS, USDA in New Orleans, LA. The ACRPC is developing an aflatoxin management program in which a key element is the treatment of large blocks of cotton acreage in Arizona with the naturally occurring AF36.

In May and June of 1999, soil samples were collected from four fields in each of ten areas from the Imperial Valley in southeastern California to the San Simon area in southeastern Arizona. The goal of the study is to develop baseline data to help assess long-term and statewide impacts of the ACRPC treatments on *A. flavus* community structure in crop soils. These sentinel sites are located at least 20 miles from *A. flavus* AF36 treatment areas. Samples are being analyzed for total *A. flavus* propagule density, the incidence of the S strain, and the incidence of AF 36.

A surface map of the incidence of the *A. flavus* S strain was generated from 1998 and 1999 data using geostatistics. The pattern shows that, on average, S strain incidence is low in eastern Arizona and high in western Arizona and southeastern California. The pattern of S strain incidence is similar to the pattern of elevation across the region. S Strain incidence in soil samples from fields over 3000 feet in elevation is consistently less than 15%. In contrast, S strain incidence in samples from fields under 2500 feet in elevation is highly variable and frequently over 50%. The trend line shows that on average S strain incidence decreases with increased elevation from 0 to 2500 ft.

The atoxigenic *Aspergillus flavus* AF36 used in the ACRPC aflatoxin management program occurs naturally in Arizona at low levels. Analysis of pre-treatment samples is not yet complete, but we have isolated AF36 from 9 of 20 fields. We anticipate isolating it from more fields as the analysis of samples continues. AF36 has been isolated from sites in the far southwestern sentinel site (Brawley, CA) and the far southeastern site (San Simon, AZ). Of the 692 *A. flavus* isolates characterized from the pre-treatment samples, fifteen (2.2%) are AF36.

ROLE OF VEGETATIVE COMPATIBILITY IN THE BIOLOGICAL CONTROL OF AFLATOXIGENIC FUNGI

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Soil populations of native *Aspergillus flavus* and *A. parasiticus* comprise numerous vegetative compatibility groups (VCGs) and these groups are widely distributed in the United States. Fungal strains within a VCG are capable of hyphal anastomosis that can result in an intermixing of nuclei to form heterokaryons. When a nonaflatoxigenic strain representing a single VCG is applied to soil for the biological control of aflatoxin contamination of crops, it may be competing with native aflatoxigenic strains belonging to the same or a different VCG. In this study, the role of vegetative compatibility in the inhibition of aflatoxin B_1 production by *A. parasiticus* was examined. Nonaflatoxigenic mutants were paired in different proportions on an agar medium with aflatoxigenic strains belonging to the same isolate, to the same VCG but with the original wild types differing in phenotype, and to different VCGs. White- and yellow-conidial color mutations also were used to detect heterokaryons and thereby indirectly measure the frequency of hyphal anastomosis. There were no consistent differences in aflatoxin B_1 inhibition by nonaflatoxigenic strains between pairings from the same VCG and pairings from different groups. In contrast, sclerotium production was significantly higher in pairings of compatible strains that formed heterokaryons than in pairings of strains from different VCGs, suggesting that the aggregate mycelium derived from hyphal anastomoses has a greater substrate base for producing sclerotia. In determining the success of a biocontrol strain for aflatoxin inhibition, strain characteristics affecting competitive ability, such as enzyme production, growth rate and capacity to survive in soil, may be more important than vegetative compatibility.

LOCALIZATION OF *ASPERGILLUS FLAVUS* AND AFLATOXIN BIOSYNTHESIS IN DEVELOPING EARS OF CORN

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Aspergillus flavus isolates transformed with the *Escherichia coli* GUS reporter gene were used to monitor fungal spread in developing corn ears. One of the isolates (GAP 2-4) was used to localize the fungus and the other isolate (GAP 26) was used to localize aflatoxin production. Ears of resistant and susceptible corn hybrids were inoculated with the GUS isolates 14 days after pollination using a modified pinbar technique. Ears were harvested at 7-day intervals until the end of the growing season. Histochemical staining was used to monitor fungal growth in kernels and cob tissues. GUS activity designating fungal presence in kernels was exclusively in the embryos. GUS activity was found more often and was much higher in susceptible cobs than in resistant cobs. Most of the GUS activity was limited to the vascular tissue of the cob. High levels of GUS activity designating aflatoxin biosynthesis were found in cobs 28 days after inoculation. High levels of GUS acitivity were also found at 49 and 56 days after inoculation. GUS activity designating aflatoxin biosynthesis was most commonly found in cob vascular tissue.

Our studies should lead to a better understanding of the infection process, fungal spread, and sites of aflatoxin biosynthesis. These results will be useful to our research program and other research programs by identifying kernel and cob tissues in resistant genotypes that most likely contain fungal growth inhibiting compounds. These studies should also provide information on the timing of assays for these growth inhibiting compounds. By identifying the actual sites of fungal inhibition and the proteins and genes associated with inhibition, the probability of transferring resistance by either conventional breeding or genetic engineering into marketable hybrids will be greatly enhanced.

POSTER PRESENTATIONS

AN *ASPERGILUS FLAVUS* NRRL 3357 MUTANT PRODUCING SYNNEMATA AND STIPITATE SCLEROTIA

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The ability to produce stipitate sclerotia and synnemata by a mutant culture derived from *Aspergillus flavus* NRRL 3357 is reported for the first time. Stipitate sclerotia were observed on Czapek agar (CZA) while synnemata formed on Murashige-Skoog agar (MSA) and on oats agar. Temperature. light and pH greatly influenced sclerotia and synnemata formation. Sclerotia were abundant on dextrose. fructose. melibiose or xylose-amended CZA whereas MSA replaced with fructose. mannitol or sorbitol produced numerous synnemata. Glycine. asparagine. or proline-amended CZA promoted sclerotia formation while cultures grown on media containing lysine. serine. or threonine produced only synnemata. Sclerotial yield was affected by the C:N ratio of the growth medium. The ability of this mutant to produce synnemata and stipitate sclerotia substantiates the view for an evolutionary link between *A. flavus* and *Stilbothamnium togoense*, a tropical fungus that produces these same structures. A single weak band was detected when the genomic DNA of *S. togoense* was fingerprinted using the DNA probe pAF28 derived from *A. flavus* suggesting some degree of homology between these two fungi.

ASPERGILLUS FLAVUS GENOTYPIC INFLUENCE ON AFLATOXIN AND BRIGHT GREENISH-YELLOW FLUORESCENCE OF CORN KERNELS

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The objective of this study was to relate the diversity of a naturally occurring population of *Aspergillus flavus* to their ability to contaminate the grain with aflatoxin and produce bright greenish- yellow fluorescent (BGYF) kernels. Nineteen strains of *A. flavus* isolated from a corn field near Kilbourne, Illinois were used as inoculum, including 16 genotypes (DNA fingerprinting), and representing both aflatoxin producers and non-producers. A commercial corn hybrid (Pioneer 3394) was grown in this field in 1996 and 1998 and twenty ears in the late milk to early dough stage of maturity were inoculated with each *A. flavus* strain using a toothpick wound procedure. At harvest, 20-24 kernels nearest each wounded site were separated into three categories: wound-inoculated kernels, intact BGYF kernels, and all other intact kernels. Sample weights of intact BGYF kernels in 1996 and 1998 grain samples averaged 5.0 % and 9.5% of the total sample weight, respectively. Aflatoxin producing strains were associated with a higher frequency ($P<0.05$) of BGYF kernels for grain samples harvested in 1998. Removal of the individual wound-inoculated kernels and the intact BGYF kernels from corn ears inoculated with 13 aflatoxin-producing strains of *A. flavus*, lowered mean aflatoxin values from 115 ng/g (range = < 1 to 387 ng/g) to 2 ng/g for 1996 grain samples and from 744 ng/g (range = 20 to 1416 ng/g) to 33 ng/g for 1998 grain samples. Results indicated substantial variation among *A. flavus* genotypes in their ability to produce aflatoxin in the germ and endosperm of infected BGYF kernels. The naturally occurring *A. flavus* population may include a majority of strains that produce no aflatoxin but exhibit BGYF and are thus aflatoxin "false positives" when corn grain is examined with an ultra violet light at 365 nm. Intraspecfic competition between aflatoxin producing and non-producing strains would be expected to naturally suppress the severity of aflatoxin outbreaks within the Midwestern corn belt.

CHARACTERIZATION OF THE *ASPERGILLUS FLAVUS* POPULATION FROM A CALIFORNIA TREE NUT ORCHARD

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Both domestic and export markets of almonds, pistachios and walnuts presently allow a maximum level of aflatoxin B_1 level in the edible nuts to be less than 20 ppb. Aflatoxin levels of 4 ppb or lower in nuts have been set by importing European countries. Even a very low degree of infection by *A. flavus* can result in aflatoxin levels of the nuts above these mandatory standards. California is the major state in the United States for the production of almonds, pistachios and walnuts, with a total market value approaching two billion dollars. Reduction of *A. flavus* population in orchards may be useful to decrease infection and thus the aflatoxin content in edible nuts. An understanding of the genotype of *the A. flavus* population in orchard environments will provide important information on the source of fungal infection.

Strains of *A. flavus* were isolated from nut fruits, nuts, flowers, leaves, stems. Air samples were collected from the vicinity of nut trees by using an MAS 100 air sampler in the month of August and September this year. The air sampler pumps 100 liters of air per minute. Spores of *A. flavus* were detected in one to five minute sampling time.

All the strains used in this study were isolated in 1998 and purified as single colonies, but only the strains of *A. flavus* associated with pistachios were characterized. The repetitive DNA probe, pAF28, cloned from *A. flavus* genome was used to define the genotypes. The probe correlates well with different VCG groups. Preliminary assignment of VCGs to the isolate was based on the pattern of Southern hybridization of the total genomic DNA to the probe. Aflatoxin B_1 production by each isolate was determined by HPLC analysis in a HP Chemstation. Strains which produced sclerotia on PDA was noted.

Twenty five-fingerprint groups representing possibly different VCGs were identified. Eight VCGs had two or more isolates. Five isolates belong to genotype 19. Therefore, this group is the most common one in the population. However, all the five strains isolated from pistachio flowers and nut hulls were in Group 19 and were found to be atoxigenic. We suggest that this group may naturally reduce the number of toxigenic strains in the orchard. Since most of the strains do not produce any sclerotia at all, they can not be classified as L strain. Ten out of forty strains are toxigenic and produce different amounts of aflatoxin B_1 when cultured on PDA. Four of the ten toxigenic *A. flavus* were from air samples. Characterization of *A. flavus* population in orchards may be useful for predicting the severity of aflatoxin contamination in tree-nuts in any particular year.

CONTROL OF *ASPERGILLUS FLAVUS* IN CONTAMINATED ALMONDS BY SAPROPHYTIC YEASTS

Sui-Sheng T. Hua, James L. Baker and Melanie Flores-Espiritu. Western Regional Research Center, ARS, USDA, Albany, CA

Because certain saprophytic yeasts have been shown to effectively compete with post -harvest fungal pathogens, such as *Penicillium expansum* and *Botrytis cinerea* on fruits, they are used as biocontrol agents. However the potential of saprophytic yeasts to be used for reducing aflatoxin contamination in almonds has not been hitherto extensively explored. *Aspergillus flavus*, the most common aflatoxin producing fungus on corn, cotton, tree-nuts and peanuts, is a wound invading pathogen that infects plants damaged by insects, animals, early splits and mechanical harvesting. Furthermore, any established infection of *A. flavus* will result in rapid accumulation of aflatoxins in the harvested commodity under warm temperature and highly humid conditions.

The visual bioassay, using *nor* mutant of *A. flavus* as an indicator strain, provides a simple and useful monitoring system for screening large numbers of yeast strains for their effectiveness in blocking aflatoxin production. If the *nor* mutant does not produce the red orange pigment, it means that the yeast can effectively stop the aflatoxin production by the fungus. This report is the first to demonstrate that certain yeasts can interact with *A. flavus* to reduce aflatoxin levels. Applications of these effective yeasts for reducing aflatoxin contamination in almond commodity warrants further research. Several hundred yeasts were isolated from almond and pistachio orchards. Fifty of them have been screened for their biocontrol activities.

Raw almonds sold in retail stores were placed on a special medium and incubated for seven to ten days at 28^0 C. The fungi grown on the almonds were identified by microscopic evaluation and by culturing the isolated microorganism on agar media. Selected yeast used for biocontrol experiments were grown in potato dextrose broth for 24 hr at 28^0 C. The yeast cultures were diluted to 10^7 cells/ml and applied to the nuts to control the fungi.

All the nuts tested were shown to have fungi on them and almost 99% of the nuts had *A. flavus* and *A. niger*. Preliminary results indicate one out five *A. flavus* was toxigenic and produced aflatoxin B_1 on PDA. Other common fungi on the almonds are *Penicillium spp*, *Aspergillus ochraceous*, *Alternaria spp* and *Aspergillus tamarii*.

Four yeasts, 415, 8-24, 99-1 and 99-3, were tested for their capacity to control fungi on almonds. Yeasts 415 and 99-1 showed good biocontrol activities to all the common fungal species on them. However, if the nuts exhibited internal fungal infection before the application of yeasts, the growth of *A. flavus* could not be controlled. These results suggest that preharvest biocontrol by yeast may be an important strategy in efforts to reduce the population of *A. flavus* in orchards.

NATURAL COMMUNITIES OF *ASPERGILLUS* SECTION FLAVI IN THE SONORAN DESERT

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Natural communities of *Aspergillus* section Flavi could have the potential of altering the structure of communities of *A. flavus* in cultivated lands. Few studies have addressed the presence and importance of this group in natural habitats, and none have studied the spatial distribution of *Aspergillus* section Flavi in a natural setting. *Aspergillus* section Flavi is known to thrive in hot arid environments and previous studies have reported these fungi in cultivated and non-cultivated areas of the Sonoran desert. Communities of *A. flavus* in natural habitats were examined to provide insight into ecological roles of aflatoxin producing fungi and potential influences of natural habitats on aflatoxin contamination of crops. Five areas 10 square miles in size were sampled in two regions of the Sonoran desert: the Colorado and Arizona desert. The Colorado desert is a low lying desert characterized by plains dominated by creosote bush (*Larrea tridentata*), bur sage and saltbrush. The Arizona desert is a higher altitude desert dominated by large cacti including saguaros, chollas and prickly pears and with creosotes in the lower bajadas. Two of the sampled areas were in close proximity to (1 to 3 mi.) and three areas were distant (>10mi.) from agriculture. Within each area, soil, plant debris and animal dung were collected periodically from multiple sites.

Colony forming units of *Aspergillus* section Flavi were estimated from platings on modified rose bengal agar. Individual colonies were isolated and characterized by species and/or strain. *Aspergillus* section Flavi was found in 291 of 345 samples analyzed (limit of detection = 3 CFU/g). Members of the section were detected in all sites, at all locations and on all substrates sampled, indicating the ubiquitous nature of this group in the Sonoran desert. Only two species within section Flavi were found, *A. tamarii* and *A. flavus*. However, both the S and L strains of *A. flavus* were present. Plant and dung samples had on average 10-fold more propagules/g than soil. In addition 98% of plant samples yielded *Aspergillus* section Flavi compared to 81% and 62% of dung and soil samples respectively. The overall incidence of *A. tamarii* was 14%, however it ranged from 0% to 44% and was generally higher in the Arizona desert. The overall incidence of *A. flavus* strain S was 9%, ranging from 0% to 34%. The incidence of the S strain was highest in the Colorado desert and it was rare (< 0.5%) in the Arizona desert sites.

In order to estimate the density and distribution of *Aspergillus* section Flavi on a smaller spatial scale, samples were also collected from four 50X50' grids. Point soil samples (≈ 1g) were taken at 5' intervals for a total of 100 samples per plot. Colony forming units were estimated and isolates characterized. Analysis of plot samples (400 total) showed presence of *Aspergillus* section Flavi in 99% of the samples. Colony forming units ranged from 0 (limit of detection = 1 CFU/g) to 97 CFU/g with an average of 11 CFU/g. The distribution of *Aspergillus* section Flavi on a small spatial scale was characterized by pockets of high propagule concentration adjacent to pockets of low propagule concentration. These adjacent high and low propagule pockets could not be explained by the presence of vegetation which had no correlation with either CFU or the percentage of the S strain.

DIFFERENCES IN THE OCCURRENCE OF ASPERGILLUS FLAVUS IN PEANUT IN MALAWI TESTS OF CULTIVARS AND LOCATIONS.

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The project on groundnut in Malawi was initiated in the 1997-1998 growing season. The market survey was initiated in Malawi in 1997-1998, the first year 29 groundnut samples were collected from vendors and farmers. No highly contaminated samples were found in the market, the aflatoxin content ranged from non detectable to 4 ppb total aflatoxins. Peanut genotypes commonly grown and promising ICRISAT genotypes were grown at three locations in Malawi in a randomized complete block design. Many yield and quality factors were measured as well as aflatoxin and Aspergillus flavus. The pea nuts were separated into those with no damage, mechanically damaged, termite scarified, immature and rotted before analysis for aflatoxins and mycoflora. No high amounts of aflatoxins were found in any of the good peanuts in 1997-98. In future years the damaged peanuts will be recombined and aflatoxin measurements made on these high risk components. There was a significant difference in Aspergillus flavus incidence in peanut genotypes in some of the locations in Malawi. This study was repeated in 1998-1999 and it will be interesting to see if this relationship holds up.

REGULATION OF AFLATOXIN BIOSYNTHESIS

POSTER PRESENTATIONS

CHARACTERIZATION OF *avfA* INVOLVED IN THE CONVERSION OF AVERUFIN AND *omtB* IN THE CONVERSION OF DEMETHYLSTERIGMATOCYSTIN AND DIHYDRODEMETHYLSTERIGMATOCYSTIN INVOLVED IN AFLATOXIN BIOSYNTHESIS IN *ASPERGILLUS PARASITICUS* AND *ASPERGILLUS FLAVUS*

Jiujiang Yu[1], Charles P. Woloshuk[2], Deepak Bhatnagar[1], and Thomas E. Cleveland[1]; [1]Southern Regional Research Center, USDA, Agricultural Research Service, New Orleans, LA and [2]Department of Plant Pathology, Purdue University, West Lafayette, IN

Aflatoxin (B_1, G_1, B_2, and G_2) biosynthesis is a multi-enzyme process controlled genetically by over 20 genes. In this report, we have identified two genes in *Aspergillus parasiticus*: *omtB*, encoding *O*-methyltransferase B for the conversion of demethylsterigmatocystin (DMST) to sterigmatocystin (ST) and dihydrodemethylsterigmatocystin (DHDMST) to dihydrosterigmatocystin (DHST); and *avfA*, involved in the conversion of averufin (AVF) to versiconal hemiacetal acetate (VHA). Both the *omtB* and *avfA* genes from *A. parasiticus* were found to be expressed in the aflatoxin-conducive media (Glucose medium solution, GMS), but not in non-aflatoxin-conducive media (Peptone medium solution, PMS). Sequence comparisons among the Aspergilli demonstrated that the DNA region including *omtB* and *avfA* genes in *A. parasiticus* are highly homologous to the counterpart genes in *A. flavus*, and *A. sojae* with overall identities about 96%, 99% respectively. The *omtB* and *avfA* genes in *A. parasiticus* are homologous to *stcP* and *stcO* in *A. nidulans* with overall identities 75% and 55%, respectively. Complementation of an averufin-accumulating mutant strain of *A. parasiticus* SRRC 165 with the *avfA* homolog from *A. flavus*, restored its ability to convert AVF to VHA and to produce aflatoxins B_1, G_1, B_2 and G_2. Sequence analysis revealed that a single amino acid replacement from aspartic acid to asparagine disabled the function of the enzyme in the mutant strain SRRC 165.

SUGAR UTILIZATION GENE CLUSTER MARKS ONE END OF THE AFLATOXIN PATHWAY GENE CLUSTER IN *ASPERGILLUS PARASITICUS*

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Aflatoxins are potent carcinogenic secondary metabolites produced by the fungi *Aspergillus parasiticus* and *A. flavus* in food and feed. Aflatoxin biosynthesis is a complex process involving over 20 biochemical reactions, preferring simple sugars (hexoses) as the carbon source. In our efforts to elucidate the genetics of aflatoxin production, we have found that genes responsible for toxin synthesis are clustered on a 70 kb piece of DNA. The genes have been established to be under the regulation of *aflR* gene which is also located on this cluster. At one end of this aflatoxin synthesis gene cluster, we have cloned another group of 4 genes (15 kb) that are apparently involved in sugar utilization by the fungi. One of the four genes, named *hxtA*, encodes for a hexose transport protein. A hydrophobicity plot of the amino acid sequence indicates that this protein contains 12 membrane spanning regions responsible for uptake of hexose such as glucose and galactose. This *hxtA* gene was found to be expressed in aflatoxin conducive growth medium (a medium containing glucose or sucrose) and no expression was detected in non-aflatoxin conducive medium (a medium containing peptone instead of glucose or sucrose as the sole carbon source). On the basis of expression pattern, the hexose transport protein appear to be genetically and biologically linked to aflatoxin production.

REGULATION OF *AFLR* AND *AFLJ*

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aflJ resides within the aflatoxin biosynthetic gene cluster adjacent to the pathway regulatory gene *aflR*. *aflJ* is involved in aflatoxin production, but its function is unknown. In an effort to study the function and regulation of *aflJ*, *Aspergillus flavus* aflatoxin-producing strain 86-10 and a strain lacking the entire biosynthetic cluster, 649-1, were transformed with either reporter constructs, expression constructs, or cosmid clones, and analyzed for gene expression or metabolite accumulation. Transformants of 86-10 harboring the overexpression construct *gpdA::aflJ* had elevated but glucose regulated aflatoxin production. To determine if this upregulation was the result of increased transcription of aflatoxin pathway genes, strain 649-1 was transformed with *gpdA::aflJ* and constructs in which the GUS reporter gene was driven by promoters of the pathway genes, *ver-1*, *omtA* and *aflR*. Overexpression of *aflJ* did not result in elevated transcription of *ver-1*, *omtA* or *aflR*. To determine if overexpression of *aflJ* leads to an increase in early pathway intermediates, strain 649-1 was transformed with cosmid 5E6 and either *gpdA::aflJ* alone, *gpdA::aflR* alone, or *aflJ* and *aflR* together. Cosmid 5E6 contains the genes *pksA*, *nor*-1, *fas-1*, and *fas-2*, that are required for the biosynthesis of the early pathway intermediate averantin. Transformants containing 5E6 alone produced no detectable averantin. In contrast, 5E6 transformants with *gpdA::aflR* produced averantin but only half as much as those transformants containing *aflR* and *aflJ*. Northern blot analysis showed that 5E6 transformants containing both *aflR* and *aflJ* had five times more *pksA* transcripts and four times more *nor-1* transcripts than 5E6 transformants containing *gpdA::aflR* alone. Further, we found that *aflJ* transcription is regulated by *aflR*. Overexpression of *aflR* resulted in elevated *aflJ* transcription.

CHARACTERIZATION OF THE AFL1 LOCUS IN ASPERGILLUS FLAVUS BY AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) ANALYSIS

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Aflatoxins are secondary metabolites produced by Aspergillus flavus, a fungus which infects corn, peanuts, cotton and tree nuts. While aflatoxins serve an unknown function in the fungus, they are highly toxic and carcinogenic to mammalian species. A majority of the genes involved in aflatoxin biosynthesis are contained within a cluster in the genome. The A. flavus strain 649 contains a deletion for this gene cluster and is unable to produce aflatoxin. Interestingly, when 649 is crossed to a wild type strain which is capable of aflatoxin production, the resulting diploid does not produce aflatoxin. To better understand this phenomena, an attempt was made to map the deleted region of 649, with particular interest in identifying the junctions. Library walks identified holes in both cosmid and BAC genomic libraries. An alternative approach, amplified fragment length polymorphism (AFLP) analysis was used to compare progeny from a parasexual cross between two strains, 86 (wild type) and 649 (afl1 deletion), which were isogenic except for the afl1 locus. Wild type and deletion progeny were screened with 103 different primer pairs. Of the 5396 bands scored, 19 of these were unique to the wild type progeny. Thirteen of these bands were used as probes on Southern blots of previously identified cosmid clones which had been mapped to the afl1 locus. One AFLP (AFLP 12) failed to hybridize to any of the clones. Further analysis will involve characterizing this DNA fragment to determine its position in relationship to the afl1 locus.

STIMULATION OF AFLATOXIN BIOSYNTHESIS IN *ASPERGILLUS PARASITICUS* BY cAMP ANALOGS

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Genetic and biochemical data suggest that the biosynthesis of aflatoxin by *Aspergillus parasiticus* is closely associated with fungal morphogenesis. We have shown that cAMP analogs, N^6,2'-O-dibutyryladenosine 3':5'-cyclic monophosphate (DcAMP) or 8-bromoadenosine 3':5'-cyclic monophosphate (BrcAMP), exogenously added, significantly increase asexual sporulation (up to10 fold) and aflatoxin accumulation (up to 20 fold) in *Aspergillus parasiticus* D8D3. DMSO, chelerythrine, H89, PD98059 (which affect cAMP signaling) differentially affected morphogenesis and aflatoxin but did not block the stimulatory effect of DcAMP on aflatoxin production. The presence of DcAMP resulted in elevated transcription of the *nor*-1 (an aflatoxin biosynthetic gene) promoter as measured by β-D-glucuronidase activity (GUS fused to the *nor*-1 promoter). In D8D3 the effect of DcAMP to increase aflatoxin production in the fungus, may be in part explained by a decrease in the level of DNA-binding protein interaction in the *nor*-1 promoter. We suggest that the signaling pathway (or parallel pathways) for the regulation of morphogenesis and aflatoxin biosynthesis diverge and that cAMP analogs act at a common part of the pathway.

UNDERSTANDING THE HOW AND WHY OF AFLATOXIN BIOSYNTHESIS FOR TOXIN ELIMINATION IN CROPS

Deepak Bhatnagar, Jeffrey W. Cary, Perng-Kuang Chang, Thomas E. Cleveland, Kenneth C. Ehrlich, and Jiujiang Yu; USDA/ARS/SRRC, New Orleans, LA

The complete characterization of aflatoxin genes and their regulation has not only been extremely beneficial in our understanding of how and why the toxin is produced by the fungus when it invades a crop, but has also aided in the success of other projects seeking to develop non-aflatoxigenic biocompetitive fungi or to monitor crop resistance to fungal growth and aflatoxin formation.

THE "HOW" OF AFLATOXIN SYNTHESIS

*Studies were conducted on a region of DNA in the fungus which houses all the genes encoding enzymes that catalyze the chemical reactions leading to aflatoxin formation. Twenty genes were discovered which were found to be involved in aflatoxin production. A regulatory gene and a new gene encoding a membrane bound secretory protein were discovered and characterized. The latter gene could be involved in aflatoxin secretion or transport and thus would provide a "target" for aflatoxin inhibition. The "master switch" gene (aflR) was discovered in the same region that governs aflatoxin production and which could be targeted essentially to switch off aflatoxin production. The regulatory gene function has been elucidated in significant detail. Through studying a non-toxigenic strain in the *A. flavus* group (*A. sojae*), a fungus used in industrial fermentations, it was discovered that the master gene in this fungal strain contained a premature stop codon leading to premature termination near the carboxy terminus of this regulatory protein (AFLR). Thus, with the absence of the functional regulatory protein, no induction of aflatoxin can occur in these food grade *Aspergilli*, even though all other aflatoxin pathway genes appear to be present and functional.

*The molecular basis for effects of nitrogen and carbohydrates sources on toxin synthesis was examined by cloning the gene clusters for nitrogen and sugar metabolism in aflatoxigenic fungi.

*A relationship between fungal development (for example, production of reproductive structures such as spores and sclerotia (survival structures) and aflatoxin synthesis was studied. Knowledge of fungal development and associated aflatoxin inductive mechanisms could lead to a dual strategy to destroy both the fungus' reproductive survival and aflatoxin producing capabilities.

*Research to disrupt genes in the aflatoxin gene cluster and convert aflatoxin producing strains to non-producing strains resulted in development of effective methodology for this conversion. These "disarmed" aflatoxin non-producing strains could be applied to the field to out compete toxin producing strains or used in industrial applications.

*Reporters genes were engineered in the laboratory and introduced into the fungus for rapid assessment of aflatoxin gene expression when plants are invaded by the fungus.

***Utility*:**

I. a. Study of the mechanism of transciptional regulation of genes in the fungus affecting aflatoxin synthesis will help in identifying natural proteins/chemical modulators and environmental factors that influence aflatoxin production. Based on acquired knowledge of molecular

regulation of toxin synthesis, feasibility of selectively inhibiting aflatoxin gene expression can be determined.

b. The reporter gene contructs can be utilized, under specific aflatoxin pathway gene promoter control, to identify environmental and nutritional signals in the plants, and their sites of synthesis in specific plant tissues, that are important in governing the degree of aflatoxin production. Specifically, further experiments can now be conducted to elucidate how environmental factors (fungal growth substrates, host plants, etc.) influence the genetic regulation of aflatoxin biosynthesis; to utilize reporter gene assays based on tester fungi containing aflatoxin genes/promoter-reporter gene constructs to assess influence of plant biochemicals on aflatoxin gene expression during the host plant *A. flavus* interaction; to determine the effect of selected plant volatiles derived from the plant lipoxygenase pathway on fungal development, reproduction, and sporulation, processes which are critical to fungal survival and which share genetic linkages with the aflatoxin biosynthetic process. These will complement the host-resistance (**plant breeding/ genetic engineering) aspects of aflatoxin control.**

II. Using pertinent genes/probes identified in previous studies, the molecular basis for the phenomenon of "natural" non-production in certain members of the *A. flavus* group, and the stability of such characteristics can be determined. Effects of individual gene disruptions on aflatoxin biosynthesis, infectivity of disrupted strain in plants and other morphological or phenotypic effects of gene disruption in the strain can be accessed. **This will ensure the safety of biological control strains.**

THE "WHY" OF AFLATOXIN SYNTHESIS

(1) **Is aflatoxin an antibiotic?** Aflatoxins are considered weak antibiotics. However, it is possible that may have been antibiotics against certain enemies that have now been completely eliminated.

(2) **Is aflatoxin anti-insects?** There is evidence that aflatoxin can be toxic to insects, but at concentrations much higher than routinely found in nature. Moreover, the fungus uses insect exit holds to infect the crops. The most probable insect-fungal interaction seems to be that insects are ideal vectors for *A. flavus*.

(3) **Does aflatoxin give any competitive advantage in the infection process?** Aflatoxigenic strains can infect crops just as effectively as toxigenic strains. This infectivity has been assigned to a unique pectinase. But it is yet to be determined if the expression of pathway genes in the atoxigenic strains (without toxin production) also contributes to the ability of fungal strains to infect crops.

(4) **Does aflatoxin play a role in fungal survival?** Fungal development and toxin synthesis are shown to be corregulated. But atoxigenic strains do develop normally and multiply.

(5) **Is aflatoxin pathway a carbon sink?** Earlier it was believed that as growth stops, all the excess carbon in fungal cells is gotten rid of by the fungus as secondary metabolites (in this case aflatoxins). With the discovery of the aflatoxin biosynthetic pathway, it seems unlikely that the fungus maintains such a complex, organized mechanisms and spends so much energy to carry out such a simple task. However, this is a plausible reason for production of aflatoxins because higher organisms do use complex mechanism to rid themselves of undesirable molecules.

(6) **Is the genetic complement for aflatoxin production a common trait amongst fungi?** Several Aspergilli produce aflatoxins whereas others do not. In addition, various other unrelated fungi, while not producing aflatoxins, either produce pathway metabolites (indicating presence of pathway genes) or do not produce any metabolites but contain genes required for toxin synthesis.

Fungi that contain aflatoxin pathway genes

Fungal species	Aflatoxin pathway Metabolite produced
Aspergilli	
A. flavus	AF
A. parasiticus	AF
A. nomius	AF
A. tamarii	AF
A. ochraceoroseus	AF
A. nidulans	ST
A. versicolor	ST
A. ustus	ST
A. variecolor	ST
A. quadrilineatus	ST
A. aurantis - brunereus	ST
A. sojae	(None)
A. oryzae	(None)
Other	
Bipolaris sorhiniana	ST
Chactomium spp.	OMST
Fusarium spp.	OMST
Monocillium sp.	OMST
Dothistioma pimi	?
Leptosphaeria maculans	?
Sorlorina crocea (lichen)	NOR

***Utility*:**

Although no role for aflatoxins in the survival of the fungus is obvious, it may have in the past or even now serve as a deterrent to enemies of the fungus. Thus, aflatoxin biosynthesis may have some evolutionary significance. Understanding why some fungi (Aspergilli and non-Aspergilli) continue to make aflatoxins (or its precursors) and why other fungi have lost the ability to make these toxins (even though they contain the genes, and are surviving in the nature) will enable us to find ways to prevent the contaminating Aspergilli from making toxins. Also, we may be able to follow the lateral transfer of genes between related and unrelated fungi to evaluate the field safety of biocontrol strains.

DETECTION, ANALYSIS, AND EXTRACTION OF AFLATOXINS

POSTER PRESENTATIONS

RAPID AND SENSITIVE ANALYTICAL METHODS FOR ALFATOXINS: AN UPDATE

Don Wauchope[1], Annie Ho[2], Corley Holbrook[1], Brad Haney[3], Chris Hassapis[4], Wellington Mubatanhema[2], David Wilson[2]; [1]USDA-ARS, Tifton, GA, [2]Univ. of GA, Tifton, [3]Blue Diamond Growers, Sacramento, CA, and [4]Saddleback Aerospace Corp.

FILIA ANALYSIS FOR AFLATOXIN B1

FILIA (Flow-Injection Liposomal ImmunoAssay) appears to be a good candidate technique for rapid, portable and inexpensive analysis of aflatoxins. Liposomes were successfully "tagged with aflatoxin B1; however, aflatoxin B1 is so hydrophobic it disrupted liposome formation. This problem has been solved by using a longer "bridge" molecule. A miniaturized, automated procedure called MFILIA is being developed by Saddleback Aerospace under a USDA Small Business Innovation Grant--see http://www.saddle-aero.com/minchem.htm.

LIPOSOMAL DIP-STRIP TEST FOR AFLATOXIN B1

Using the tagged liposomes from the FILIA experiment, it is possible to develop a simple "dip-stick" procedure in which plastic-backed nitrocellulose paper strips behave just like the columns, but the dye is simply released in place and the intensity read by eye (or densitometer for more quantitative results). Our first test gave a useful range from about 10 to 10,000 ppb aflatoxin B1.

SPE COLUMNS FOR AFLATOXIN ANALYSIS AND REMOTE SAMPLING

Standard SPE (solid phase extraction) columns gave comparable results to immunosorbent columns as an isolation technique prior to HPLC analysis. In addition, pesticides sorbed on SPE disks have been shown to be quite stable, and, once dry, such disks can be sent via regular mail to distant laboratories for analysis. SPE columns with adsorbed aflatoxins were spiked with standard aflatoxins and shipped from Blue Diamond Growers Labs in Sacramento to Tifton and analyzed with 80% or better recoveries on arrival. This suggests a convenient and inexpensive way to preserve and ship aflatoxin samples for remote analysis.

IDENTIFICATION OF THE BRIGHT-GREENISH-YELLOW-FLUORESCENCE (BGY-F) COMPOUND ON COTTON LINT ASSOCIATED WITH AFLATOXIN CONTAMINATION IN COTTONSEED

Hampden J. Zeringue, Jr.[1], Betty Y. Shih[1], Karol Maskos[2], and Deborah Grimm[2]. [1]USDA, ARS, SRRC, P. O. Box 19687, New Orleans LA 70179-0687, [2]Tulane University, 605 Lindy Boggs Building, New Orleans, LA 70118

In order to characterize the structure of the bright-greenish-yellow-fluorescence (BGY-F) compound on cotton lint associated with aflatoxin contamination in cotton seed, various *in vitro* and *in vivo* natural BGY-F reaction products were prepared. Under similar high pressure liquid chromatography separation with variable wavelength and programmable fluorescence detection (HPLC-UV/FL), combined with atmospheric pressure ionization and mass spectral determinations it was found that the BGY-F reaction products prepared from three preparations: (a) kojic acid (KA) + peroxidase (soybean peroxide or horseradish type VI and type II) + H_2O_2, or (b) detached fresh cotton locules + KA + H_2O_2, or (c) attached field cotton locules that were treated with a spore suspension of aflatoxigenic *Aspergillus flavus*, all resulted in identical chromatographic characteristics. Also these three reaction products exhibited a molecular weight of 282 for the BGY-F compound. Further characterization of the BGY-F reaction product with ^1H and ^{13}C NMR analysis indicated that it was a dimer of 2 KA, minus two protons, linked at the C-6 positions.

SPECIAL PRESENTATION

DEVELOPMENT OF EXPERT SYSTEMS FOR THE UNITED STATES PEANUT INDUSTRY

M.C. Lamb[1], J. I. Davidson, Jr.[2], and D. A. Sternitski[3]; [1]Agricultural Economist, [2]Mechanical Engineer, [3]Agricultural Engineer, USDA-ARS National Peanut Research Laboratory, Dawson, GA

Expert systems are being developed to facilitate technology transfer in the U.S. peanut industry. Expert systems offer an effective technology transfer tool because as agriculture becomes increasingly complex the demand for more effective management methods increases. The USDA-ARS National Peanut Research Laboratory has developed several expert systems specific to certain aspects of peanut production which operate independently and the integration of these systems into an integrated peanut management system is being conducted. Several expert systems were discussed. Farm Office is a whole farm planning and crop rotation optimization system which can be used to develop optimized farm plans. Reduction in the per ton value in peanuts equates to approximately $430 per ton and corn could be reduced through the inability to market contaminated corn. During drought years aflatoxin detection of both peanut and corn lots can imposed significant reductions in farm income and is often the difference in obtaining positive or negative farm income. The economic impact of aflatoxin contamination was analyzed with a case farm example. EXNUT (also known as Irrigator) is an expert system designed to manage peanut irrigation. EXNUT uses new concepts such as geocarposphere temperature, total water, soil type, plant maturity stage, and other to recommend the timing and amount of peanut irrigation. The benefits of EXNUT directly impact both the farm and non-farm sectors. Yield and grade increases of 500 to 700 pounds per acre and 2-5%, respectively, have been documented. Combine with the low cost of running EXNUT ($3.71 per acre), this equates to a net return to EXNUT of $92.56 per acre. Laboratory analyses indicated that no aflatoxin was found in samples from the EXNUT managed fields. Further, a 4 to 6 acre/inch annual reduction in irrigation from EXNUT was documented with farmer comparisons. MNUT is an expert system designed to reduce risk to various peanut industry segments involved with marketing peanuts. MNUT utilizes similar concepts as EXNUT to provide objective predictions of yield, grade, germination, shelling characteristics, and aflatoxin at weekly intervals during the growing season. This allows improved segregation of farmer stock peanuts to prevent commingling of peanuts which improves processing efficiency at peanut shelling plants. Expert systems offer an effective tool to expedite technology transfer of data and information to the United State peanut industry. A CRADA (Cooperative Research and Development Agreement) has been enacted with The Peanut Foundation to manage the release and distribution of the expert systems.

AGENDA
1999 AFLATOXIN ELIMINATION WORKSHOP
OCTOBER 20-22, 1999
ATLANTA, GA

WEDNESDAY, OCTOBER 20, 1999

5:00-8:00 p.m. **REGISTRATION/POSTER ASSIGNMENTS**

6:00 p.m. **POSTER VIEWING**
(Posters will be available for viewing for the duration of the meeting)

6:00-8:00 p.m. **MIXER**

THURSDAY, OCTOBER 21, 1999

7:00 a.m. **REGISTRATION/POSTER ASSIGNMENTS**

8:00 a.m. **WELCOME:** Howard Valentine, American Peanut Council

INTRODUCTORY REMARKS: Jane F. Robens
USDA, ARS, National Program Staff

SESSION 1: CROP RESISTANCE- CONVENTIONAL BREEDING
Chair: Henry L. Keller, Lone Star Corn Growers Assoc.

8:15 a.m. **"Eliminaton of aflatoxin contaminated BGYF kernels through conventional breeding"**, Donald T. Wicklow[1] and Lori C. Marshall[2]. [1]USDA, ARS, National Center for Agricultural Utilization Research, Peoria, IL; [2]Holden's Seeds, Williamsburg, IA.

8:30 a.m. **"Endocarp ventral vascular tissue development appears to be the Achilles heel for almond susceptibility to insect damage and aflatoxin contamination"**, Thomas M. Gradziel. University of California-Davis, Davis, CA.

8:45 a.m. **"Evaluation of mutant B73 and A632 corn inbreds for aflatoxin reduction and agronomic traits"**, Charlie A. Martinson. Iowa State University, Ames, IA.

9:00 a.m. **"Inheritance of, molecular markers associated with, and breeding for, resistance to Aspergillus ear rot and aflatoxin production in corn"**, Donald G. White, Torbert R. Rocheford, Gnanambal Naidoo, Chandra Paul, Rebecca D. Rozzi, Dina E. Severns, and Amy M. Forbes. Universtiy of Illinois at Urbana-Champaign, Urbana, IL.

9:15 a.m. **"Pyramiding ear resistance to insects and invasion by *Aspergillus* spp. for control of preharvest aflatoxin contamination"**, Neil W. Widstrom,[1] Ana Butron,[1] Baozhu Guo,[1] David M. Wilson,[2] Maurice E. Snook,[2] and Thomas E. Cleveland.[3] [1] USDA-ARS, Insect Biology and Population Management Research Lab, Tifton, GA; [2] University of Georgia, Coastal Plain Exp. Station, Tifton, GA; [3] USDA-ARS, Southern Regional Research Center, New Orleans, LA.

9:30 a.m. **"RFLP markers associated with silk antibiosis to corn earworm"**, Baozhu Guo[1], Zhongjun Zhang,[2] Maurice E. Snook,[2] Neil W. Widstrom,[1] Patrick F. Byrne,[3] Michael D. McMullen,[4] and Robert E. Lynch[1]. [1] USDA-ARS, Insect Biology and Population Management Research Lab, Tifton, GA; [2] University of Georgia, Coastal Plain Exp. Station, Tifton, GA; [3]Colorado State University, Fort Collins, CO; [4]USDA-ARS, Plant Genetics Research Unit, Columbia, MO.

9:45 a.m. **"Resistance to aflatoxin accumulation in maize inbreds selected for ear rot resistance in West and Central Africa"**, Robert L. Brown[1], Thomas E. Cleveland[1], Zhiyuan Chen[2], Abebe Menkir[3], Kitty Cardwell[3], Jennifer Kling[3], and Donald G. White[4]. [1]USDA, ARS, Southern Regional Research Center, New Orleans, LA; [2]Louisiana State University, Baton Rouge, LA; [3]International Institute of Tropical Agriculture, Ibadan, Nigeria; [4]University of Illinois at Urbana-Champaign, Urbana, IL.

10:00-10:30 a.m. **BREAK**

10:30 a.m. **"The search for an indirect selection tool for resistance to preharvest aflatoxin contamination in peanut"**, C. Corley Holbrook[1], Craig K. Kvien[2], Kim K. Franke[2], Keith T. Ingram[2], R. Donald Wauchope[1], Huiqin Xue[3], Michael E. Matheron[4], and David M. Wilson[2]. [1]USDA-ARS, Coastal Plain Exp. Station, Tifton, GA; [2]University of Georgia, Tifton, GA; [3]Shandong Peanut Research Inst.; [4]University of Arizona, Yuma, AZ.

10:45 a.m. **"Drought and drought resistance effects on *Aspergillus flavus* infection as observed in situ with a minirhizotron camera"**, Keith T. Ingram[1], George Pateña[1], and C. Corley Holbrook[2]. [1]University of Georgia, Griffin, GA;[2]USDA, ARS, Coastal Plain Exp. Station, Tifton, GA.

11:00-11:45 a.m. **PANEL DISCUSSION**
Panel Chair: Charlie A. Martinson

11:45- 1:00 p.m. **LUNCH**

1:00-1:30 p.m. **SPECIAL LECTURE:**
"Development of Expert Systems for the Peanut Industry" Marshall C. Lamb and James I. Davidson Jr. USDA,ARS, National Peanut Research Lab, Dawson, GA.

SESSION 2: CROP MANAGEMENT AND HANDLING, INSECT CONTROL AND FUNGAL RELATIONSHIPS
Chair: Merle Jacobs, Almond Board of California

1:30 p.m. **"Aflatoxin control in figs: cultural practices and ecological relationships"**, Mark A. Doster. University of California, Davis, Kearney Agricultural Center, Parlier, CA.

1:45 p.m. **"Aflatoxin control in pistachios: reduction of early hull splitting, decreasing navel orangeworm infestation, and identifying contaminated nuts"**, Themis J. Michailides, University of California, Davis, Kearney Agricultural Center, Parlier, CA.

2:00 p.m. **"Post-harvest selection and sorting for aflatoxin in tree and ground nuts"**, Thomas F. Schatzki and Tom C. Pearson. USDA, ARS, Western Regional Research Center, Albany, CA.

2:15 p.m. **"The status of official AOAC international, AOAC research institute certified, and FGIS protocol methods for aflatoxin determination as they relate to current United States and European Union aflatoxin regulations for grain, oilseed, cottonseed, and tree nuts"**, David M. Wilson[1] and Mary W. Trucksess[2]. [1]University of Georgia, Tifton, GA; [2]FDA, Washington DC.

2:30-3:00 p.m. **PANEL DISCUSSION**
Panel Chair: Mark A. Doster

3:00-3:30 p.m. **BREAK**

SESSION 3: POTENTIAL USE OF NATURAL PRODUCTS FOR PREVENTION OF FUNGAL INVASION AND/OR AFLATOXIN BIOSYNTHESIS IN CROPS
Chair: Howard Valentine, American Peanut Council

3:30 p.m. **"Tree nut-aflatoxin interactions: natural products affecting insect pests, growth of *Aspergillus*, aflatoxigenesis and aflatoxin biotransformation"**, Bruce Campbell, Sung-Eun Lee, Douglas Light, Noreen Mahoney, Glory Merrill, Russell Molyneux, and James Roitman. USDA, ARS, Western Regional Research Center, Albany, CA.

3:45 p.m. **"Transmission of aflatoxin B1 inhibition in mycelia of *Aspergillus flavus* and other inhibitor studies"**, Robert A. Norton, USDA-ARS, National Center for Agricultural Utilization Research, Peoria, IL.

4:00 p.m. **"Linoleic acid and linoleic acid derivatives regulate *Aspergillus* development and mycotoxin production"**, Nancy P. Keller, Texas A&M University, College Station, TX.

4:15 p.m. **"Alpha amylase from *Aspergillus flavus*: inhibitors found in plant seeds"**, Charles Woloshuk, Purdue University, West Lafayette, IN.

4:30 p.m. **"Genetic studies on the regulation of aflatoxin accumulation"**, Gary A. Payne, North Carolina State University, Raleigh, NC.

4:45-5:30 p.m. **PANEL DISCUSSION**
Panel Chair: Bruce Campbell

5:30-6:00 p.m. **POSTER VIEWING**

6:00 p.m. **COMMODITY BREAKOUT SESSIONS**

FRIDAY, OCTOBER 22, 1999

8:00 a.m. **ANNOUNCEMENTS**

SESSION 4: CROP RESISTANCE- GENETIC ENGINEERING
Chair: Lynn A. Jones, National Cottonseed Products Assoc.

8:15 a.m. **"Control of aflatoxin production and fungal growth by regulating G proteins"**, Paul M. Hasegawa, Maria Coca, Meena Narasimhan, Barbara Damsz, Paola Veronese, Gyung-Hye Huh, Ron Salzman, Ray A. Bressan. Purdue University, West Lafayette, IN.

8:30 a.m. **"Purification of two small peptides with antifungal activity against *Aspergillus flavus*"**, Anne-laure Moyne[1], Jana Tonder[1], Thomas E.Cleveland[2] and Sadik Tuzun[1].[1]Auburn University, Auburn, AL; [2]USDA, ARS, Southern Regional Research Center, New Orleans, LA.

8:45 a.m. **"Genetic engineering and breeding of walnuts for control of aflatoxin"**, Gale McGranahan, Abhaya M. Dandekar, Patrick Vail, Charles Leslie, Sandie Uratsu, Steven Tebbets and Matthew Escobar. University of California-Davis, Davis, CA.

9:00 a.m. **"Inhibition of fungal growth by putative transgenic cotton plants"**, Kanniah Rajasekaran, Jeffrey W. Cary, Thomas J. Jacks, Kurt Stromberg, and Thomas E. Cleveland. USDA, ARS, Southern Regional Research Center, New Orleans, LA.

9:15 a.m. **"Engineering cotton for resistance to *Aspergillus flavus*: promoters and structural genes"**, Caryl A. Chlan[1], Renee Manuel[1], Robert Bourgeois[1], Jeffrey W. Cary[2], Kanniah Rajasekaran[2], and Thomas E. Cleveland[2]. [1]University of Southwestern Louisiana, Lafayette, LA; [2]USDA, ARS, Southern Regional Research Center, New Orleans, LA.

9:30 a.m. **"Transformation of peanut with the defensive peptidyl MIM D5C"**, Arthur K. Weissinger. North Carolina State University, Raleigh, NC.

9:45-10:15 a.m. **BREAK**

10:15 a.m. **"Genetic engineering of peanut for reduction of aflatoxin contamination-progress with Bt, peroxidase, peptide D4E1, and lipoxygenase"**, Peggy Ozias-Akins, Ravinder Gill, and Hongyu Yang. University of Georgia, Coastal Plain Exp. Station, Tifton, GA.

10:30-11:15 a.m. **PANEL DISCUSSION**
Panel Chair: Kanniah Rajasekaran

SESSION 5: MICROBIAL ECOLOGY
Chair: Phillip J. Wakelyn, National Cotton Council

11:15 a.m. **"Biocontrol research to reduce aflatoxin in almonds and pistachios"**, Sylvia Hua. USDA, ARS, Western Regional Research Center, Albany, CA.

11:30 a.m. **"Long term influences of atoxigenic strain applicatons on *Aspergillus flavus* communities in commercial agriculture"**, Peter J. Cotty. USDA, ARS, Southern Regional Research Center, New Orleans, LA.

11:45 a.m. **"Production of commercially useful quantities of atoxigenic strain inoculum"**, Larry Antilla[1] and Peter J. Cotty[2]. [1]Arizona Cotton Research and Protection Council, Tempe, AZ; [2]USDA, ARS, Southern Regional Research Center, New Orleans, LA.

12:00-1:15 p.m. **LUNCH**

1:15 p.m. **"Potential of the Koji molds, *Aspergillus oryzae* and *Aspergillus sojae*, as competitive fungi in biological control of aflatoxin contamination"**, Joe W. Dorner, Richard J. Cole, and Bruce W. Horn. UDSA, ARS, National Peanut Research Laboratory, Dawson, GA.

1:30 p.m. **"Sequence of substrate utilization during corn kernel infection and aflatoxin contamination"**, Jay E. Mellon, Michael K. Dowd, and Peter J. Cotty. USDA, ARS, Southern Regional Research Center, New Orleans, LA.

1:45 p.m. **"Sentinel sites for monitoring *Aspergillus flavus* community structure in southwestern desert agricultural soils"**, Merritt R. Nelson[1], Thomas V. Orum[1], Donna M. Bigelow[1], and Peter J. Cotty[2]. [1]University of Arizona, Tucson, AZ; [2]USDA, ARS, Southern Regional Research Center, New Orleans, LA.

2:00 p.m. **"Role of vegetative compatibility in the biological control of aflatoxigenic fungi"**, Bruce W. Horn, Ronald L. Greene, and Joe W. Dorner. USDA, ARS, National Peanut Research Laboratory, Dawson, GA.

2:15 p.m. **"Localization of *Aspergillus flavus* and aflatoxin biosynthesis in developing ears of corn"**, Gary L. Windham[1], W. Paul Williams[1], Robert L. Brown[2], Thomas E. Cleveland[2], and Gary A. Payne[3]. [1]USDA, ARS, Mississippi State, MS; [2]USDA, ARS, Southern Regional Research Center; [3]North Carolina State University, Raleigh, NC.

2:30-3:15 p.m. **PANEL DISCUSSION**
Panel Chair: Gary L. Windham

3:15-3:30 P.M. **CLOSING REMARKS: Jane F. Robens**

POSTER PRESENTATIONS
1999 Aflatoxin Elimination Workshop
October 20-22, 1999
Atlanta, GA

Poster Board Number

A. MICROBIAL ECOLOGY

A-1 **"Aspergillus flavus NRRL 3357 mutant producing synnemata and stipitate sclerotia"**, Cesaria E. McAlpin USDA, ARS, National Center for Agricultural Utilization Research, Peoria, IL.

A-2 **"*Aspergillus flavus* genotypic influence on aflatoxin and bright greenish-yellow fluorescence of corn kernels"**, Donald T. Wicklow USDA, ARS, National Center for Agricultural Utilization Research, Peoria, IL.

A-3 **"Characterization of *Aspergillus flavus* population from a California tree nut orchard"**, Sui-Sheng T. Hua[1], Cesaria E. McAlpin[2], James L. Baker[1] and Crystal E. Platis[2]. [1]USDA, ARS, Western Regional Research Center, Albany, CA; [2]USDA, ARS, National Center for Agricultural Utilization Research, Peoria, IL.

A-4 **"Control of *Aspergillus flavus* in contaminated almonds by saprophytic yeasts"**, Sui-Sheng T. Hua, James L. Baker and Melanie Flores-Espiritu. USDA, ARS, Western Regional Research Center, Albany, CA.

A-5 **"Natural communities of *Aspergillus* section flavi in the Sonoran desert"**, Maria L. Boyd and Peter J. Cotty. USDA, ARS, Southern Regional Research Center, New Orleans, LA.

A-6 **"Differences in the occurrence of *Aspergillus flavus* in peanut in Malawi tests of cultivars and locations"**, Charles T. Kisyombe[1] and David M. Wilson[2]. [1]Ministry of Agriculture, Chitedze Agricultural Research Station, Lilongwe, Malawi;[2]University of Georgia, Tifton, GA.

B. CROP MANAGEMENT AND HANDLING, INSECT CONTROL, AND FUNGAL RELATIONSHIPS

B-1 **"An ultralow volume assay system for discovering and evaluating antiinsectan biomolecules"**, Patrick F. Dowd. USDA, ARS, National Center for Agricultural Utilization Research, Peoria, IL.

B-2 **"A temporal study of aflatoxin levels in Arizona and Mississippi cotton modules"**, William E. Batson, Jr., Jacobo C. Caceres and Cecilia T. Nguyen. Mississippi State University, Mississippi State, MS. ***(Withdrawn)***

B-3 **"Aflatoxin accumulation in transgenic and nontransgenic corn hybrids infested with southwestern corn borer"**, W. Paul Williams, Paul M. Buckley, Gary L. Windham, and Frank M. Davis. USDA, ARS, Mississippi State, MS.

B-4 **"Comparison of preharvest aflatoxin accumulation in Bt and non-Bt corn in Florida and Georgia"**, David M. Wilson[1], David L. Wright[2], Pawel Wiatrak[2], Don Herzog[2], G. David Buntin[3], and Dewey Lee[1]. [1]University of Georgia, Tifton, GA; [2]North Florida Research and Education Center, Quincy, FL; [3]University of Georgia, Griffin, GA.

B-5 **"Occurrence of fumonisins and aflatoxins in the south Georgia corn survey from 1996 to 1998"**, Wellington Mubatanhema[1], Zeljko Jurjevic[1], David M. Wilson[1], Bonnie D. Evans[1], Neil W. Widstrom[2], and Filmore Meredith[3]. [1]University of Georgia,Tifton, GA; [2]USDA, ARS, Tifton, GA; [3]USDA, ARS, Athens, GA.

B-6 **"Fungal successions and mycotoxins in stored Pearl Millet"**, Zeljko Jurjevic[1], David M. Wilson[1], Jeffrey P. Wilson[2], and Howard Casper[3]. [1]University of Georgia, Tifton, GA; [2]USDA, ARS, Tifton, GA; [3]North Dakota State University, Fargo, ND.

B-7 **"Profile of aflatoxin and fumonisin accumulation in North Carolina corn"**, Brian Bush, Marty Carson, Marc Cubeta, Winston Hagler, and Gary Payne.
North Carolina State University, Raleigh, NC.

C. CROP RESISTANCE- CONVENTIONAL BREEDING

C-1 **"Summary of studies using the norsolorinic acid producing *Aspergillus parasiticus* mutant to study infection and visually screen for aflatoxin resistance in corn"**, David M. Wilson[1], Neil W. Widstrom[2], Jason H. Brock[3], Wellington Mubtanhema[3], and Bonnie D. Evans[3]. [1]University of Georgia, Tifton, GA; [2]USDA, ARS, Tifton, GA; [3]University of Georgia, Tifton, GA.

C-2 **"Evaluation of corn hybrids for resistance to aflatoxin accumulation"**, W. Paul Williams, Gary L. Windham, and Paul M. Buckley. USDA, ARS, Mississippi State, MS.

C-3 **"Molecular genetic analysis of resistance to Aspergillus flavus in maize: QTL for husk tightness and silk maysin in F2:3 lines"**, Ana Butron[1], Baozhu Guo[1], Maurice E. Snook[2], Neil W. Widstrom[1], and Robert E. Lynch[1]. [1]USDA, ARS, Insect Biology and Population Management Lab, Tifton, GA; [2]University of Georgia, Coastal Plain Exp. Station, Tifton, GA.

C-4 **"Molecular genetic analysis of resistance to insects in maize: QTL for antibiotic compounds in silks"**, Zhongjun Zhang[1], Baozhu Guo[2], Maurice E. Snook[1], Neil W. Widstrom[2], and Robert E. Lynch[2]. [1]University of Georgia, Coastal Plain Exp. Station, Tifton, GA; [2]USDA-ARS, Insect Biology and Population Management Lab, Tifton, GA.

C-5 **"Evaluation of high oil vs. low oil corn hybrids for Aspergillus ear rot and aflatoxin production"**, Dina E. Severns, Rebecca D. Rozzi, Donald G. White, and Robert J. Lambert. University of Illinois at Urbana-Champaign, Urbana IL.

C-6 **"Levels of resistance to Aspergillus ear rot and aflatoxin accumulation within F1 hybrid combinations of eight maize inbred resistance sources"**, Gnanambal Naidoo, Amy M. Forbes, Torbert R. Rocheford and Donald G. White. University of Illinois at Urbana-Champaign, Urbana IL.

C-7 **"Summary of mapping QTL's associated with Aspergillus ear rot and aflatoxin accumulation in multiple populations"**, Gnanambal Naidoo, K. Campbell, Amy M. Forbes, A. Hamblin, B. Kaufmann, Chandra Paul, Dina E. Severns, Torbert R. Rocheford, and Donald G. White. University of Illinois at Urbana-Champaign, Urbana IL.

D. CROP RESISTANCE- GENETIC ENGINEERING

D-1 **"Plant regeneration from transgenic peanut lines (*Arachis hypogaea* L.) transformed with a tomato anionic peroxidase gene"**, Ravinder Gill, HongyuYang, and Peggy Ozias-Akins. University of Georgia, Coastal Plain Exp. Station, Tifton, GA.

D-2 **"Transformation of peanut with a synthetic antifungal peptide gene"**, Hongyu Yang, Ravinder Gill, and Peggy Ozias-Akins. University of Georgia, Coastal Plain Exp. Station, Tifton, GA.

D-3 **"Sequence analysis of a corn chitinase from Tex 6"**, Kenneth G. Moore, Arthur K. Weissinger, Rebecca S. Boston, and Gary A. Payne. North Carolina State University, Raleigh, NC.

D-4 **"Maize transformation for resistance to *Aspergillus flavus*"**, Chuansheng Mei, Donald G. White, and Jack M. Widholm. University of Illinois at Urbana-Champaign, Urbana, IL.

D-5 **"Characterization of an alkaline protease excreted by *Aspergillus flavus* in infected corn kernels"**, Zhiyuan Chen[1], Jeffrey W. Cary[2], Robert L. Brown[2], Kenneth E. Damann[1], Thomas E. Cleveland[2]. [1]Louisiana State University Agricultural Center, Baton Rouge, LA; [2]USDA, ARS, Southern Regional Research Center, New Orleans, LA.

D-6 **"Characterization of *cssap92*, a corn gene responsive to *Aspergillus flavus* infection"**, Richard Wilson[1], Harold W. Gardner[1], and Nancy P. Keller[2]. [1]USDA, ARS, National Center for Agricultural Utilization Research, Peoria, IL; [2]Texas A&M University, College Station, TX.

D-7 **"Use of a green fluorescent protein expressing *Aspergillus flavus* strain to assay for antifungal factors in cottonseed"**, Kurt D. Stromberg, Kanniah Rajasekaran, Jeffrey W. Cary, and Thomas E. Cleveland. USDA, ARS, Southern Regional Research Center, New Orleans, LA.

D-8 **"Drought induced transcriptional changes in peanut (Arachis hypogaea L.)"**, Ashok K. Jain and S. M. Basha; Plant Biotechnology Program, Florida A&M University, Tallahassee, FL

E. REGULATION OF AFLATOXIN BIOSYNTHESIS

E-1 **"Characterization of *avfA* involved in the conversion of averufin and *omtB* in the conversion of demethylsterigmatocystin and dihydrodemethyl-sterigmatocystin in aflatoxin biosynthesis in *Aspergillus parasiticus* and *Aspergillus flavus*"**, Jiujiang Yu[1], Charles P. Woloshuk[2], Deepak Bhatnagar[1], and Thomas E. Cleveland[1]. [1]USDA, ARS, Southern Regional Research Center, New Orleans, LA; [2]Purdue University, West Lafayette, IN.

E-2 **"Sugar utilization gene cluster marks one end of the aflatoxin pathway gene cluster in *Aspergillus parasiticus*"**, Jiujiang Yu, Perng-Kuang Chang, Deepak Bhatnagar, and Thomas E. Cleveland. USDA, ARS, Southern Regional Research Center, New Orleans, LA.

E-3 **"Signal transduction via cAMP-dependent protein kinase is involved in sterigmatocystin production and development"**, Kiminori Shimizu and Nancy P. Keller. Texas A&M University, College Station, TX. ***(Withdrawn)***

E-4 **"Regulation of aflR and aflJ"**, Wanglei Du and Gary A. Payne. North Carolina State University, Raleigh, NC.

E-5 **"Characterization of the *afl-1* locus in *Aspergillus flavus* by amplified fragment length polymorphism (AFLP) analysis"**, Marilee Ramesh and Charles P. Woloshuk. Purdue University, West Lafayette, IN.

E-6 **"The distribution of aflatoxin enzymes in *Aspergillus parasiticus* grown on solid culture"**, Ching-Hsun Chiou, Li-Wei Lee, and John E. Linz. Michigan State University, East Lansing, MI. ***(Withdrawn)***

E-7 **"Stimulation of aflatoxin biosynthesis in *Aspergillus parasiticus* by cAMP analogs"**, Ludmila Roze and John E. Linz. Michigan State University, East Lansing, MI.

E-8 **"Understanding the how and why of aflatoxin biosynthesis for toxin elimination in crops"**, Deepak Bhatnagar, Jeffrey W. Cary, Perng-Kuang Chang, Thomas E. Cleveland, Kenneth C. Ehrlich, and Jiujiang Yu. USDA, ARS, Southern Regional Research Center, New Orleans, LA.

F. POTENTIAL USE OF NATURAL PRODUCTS FOR PREVENTION OF FUNGAL INVASION AND/OR REGULATION OF AFLATOXIN BIOSYNTHESIS IN CROPS

F-1 **"Repression of GUS reporter constructs of aflatoxin biosynthetic pathway genes by phenolic compounds"**, Sui-Sheng T. Hua[1], Wanglei Du[2], Gary A. Payne[2], Melanie

Flores-Espiritu[1] and James L. Baker[1]. [1]USDA, ARS, Western Regional Research Center, Albany, CA; [2]North Carolina State University, Raleigh, NC

F-2 **"Isoform patterns of chitinase and β-1,3-glucanase in maturing corn kernels *(Zea mays* L.) associated with *Aspergillus flavus* milk stage infection"**, Cheng Ji [1], Robert A. Norton[2], Donald T. Wicklow[2], and Patrick F. Dowd[2]. [1]University of Illinois at Urbana-Champaign, Urbana, IL; [2]USDA, ARS, National Center for Agricultural Utilization Research, Peoria, IL.

F-3 **"Effects of a novel compound on expression of mycotoxin biosynthetic genes"**, Frances Trail, Luis Velasquez, Haixin Xu, and Ray Hammerschmidt. Michigan State University, East Lansing, MI.

F-4 **"Advances toward development of a host plant volatile-based attracticide for codling moths, a key pest in *Aspergillus* invasion of walnuts"**, Douglas M. Light, Kathy Reynolds, Dayananda Rajapaska, Clive Henrick, Ronald Buttery, Glory Merrill, James Roitman and Bruce Campbell. USDA, ARS, Western Regional Research Center, Albany, CA.

F-5 **"Biotransformation of AFB1 in navel orangeworm and codling moth larvae"**, Sung-Eun Lee, Douglas M. Light, and Bruce Campbell. USDA, ARS, Western Regional Research Center, Albany, CA.

F-6 **"Naturally active compounds as inhibitors of aflatoxin biosynthesis"**, Sung-Eun Lee, Noreen Mahoney, and Bruce Campbell. USDA, ARS, Western Regional Research Center, Albany, CA.

F-7 **"Effect of almond processing on viability of *Aspergillus flavus* spores"**, Noreen Mahoney, Russell Molyneux, and Paul Bayman. USDA, ARS, Western Regional Research Center, Albany, CA.

F-8 **"Use of natural flavones to control navel orangeworm, a major pest of almond"**, James Roitman. USDA, ARS, Western Regional Research Center, Albany, CA.

F-9 **"Comparison of aflatoxin production on defatted tree nuts:high anti-aflatoxigenic activity in walnuts"**, Noreen Mahoney[1], Russell Molyneux[1], Thomas Gradziel[2], Gale McGranahan[2], Bruce Campbell[1]. [1]USDA, ARS, Western Regional Research Center, Albany, CA; [2]University of California, Davis, CA.

F-10 **"Characterization of the delta-12 desaturase gene of *Aspergillus nidulans* and its relationship with spore development and sterigmatocystin production"**, Ana Calvo[1], Harold W. Gardner[2], Nancy P. Keller[1]. [1]Texas A&M University, College Station, TX; [2]USDA, ARS, National Center for Agricultural Utilization Research, Peoria, IL.

G. DETECTION, ANALYSIS, AND EXTRACTION OF AFLATOXINS

G-1 **"Rapid and sensitive analytical methods for aflatoxins: an update"**, Don Wauchope[1], Annie Ho[2], C. Corley Holbrook[1], Brad Haney[3]; Wellington Mubatenhema[2], David Wilson[2]. [1]USDA-ARS, Coastal Plain Exp. Station, Tifton, GA; [2]University of Georgia, Tifton, GA; [3]Blue Diamond Growers, CA.

G-2 **"Structure of the bright-greenish-yellow-fluorescence (BGYF) compound on cotton lint associated with aflatoxin contamination in cottonseed"**, Hampden J. Zeringue[1], Jr., Betty Y. Shih[1], Karol Mashos[2] , and Deborah Grimm[2]. [1]USDA, ARS, Southern Regional Research Center, New Orleans, LA; [2]Tulane University, New Orleans, LA.

POSTER PRESENTATIONS
AUTHOR INDEX
1999 Aflatoxin Elimination Workshop
October 20-22, 1999
Atlanta, GA

Baker, James L. (A-3, A-4, F-1)
Basha, S. M. (D-8)
Batson, Jr., William E. (B-2)
Bayman, Paul (F-7)
Bhatnagar Deepak (E-1, E-2, E-8)
Boston, Rebecca S. (D-3)
Boyd, Maria L. (A-5)
Brock, Jason H. (C-1)
Brown, Robert L. (D-5)
Buckley, Paul M. (B-3, C-2)
Buntin, G. David (B-4)
Bush, Brian (B-7)
Butron, Ana (C-3)
Buttery, Ronald (F-4)

Caceres, Jacobo C. (B-2)
Calvo, Ana (F-10)
Campbell, Bruce (F-4, F-5, F-6, F-9)
Campbell, Keith (C-7)
Carson, Marty (B-7)
Cary, Jeffrey W. (D-5, D-7, E-8)
Casper, Howard (B-6)
Chang, Perng-Kuang (E-2, E-8)
Chen, Zhiyuan (D-5)
Chiou, Ching-Hsun (E-6)
Cleveland, Thomas E. (D-5, D-7, E-1, E-2, E-8)
Cotty, Peter J. (A-5)
Cubeta, Marc (B-7)

Damann, Kenneth E. (D-5)
Davis, Frank M. (B-3)
Dowd, Patrick F. (B-1, F-2)
Du, Wanglei (E-4, F-1)

Ehrlich, Kenneth C. (E-8)
Evans, Bonnie D. (B-5, C-1)

Flores-Espiritu, Melanie (A-4, F-1)
Forbes, Amy M. (C-6, C-7)

Gardner, Harold W. (D-6, F-10)
Gill, Ravinder (D-1, D-2)
Gradziel, Thomas (F-9)
Grimm, Deborah (G-2)
Guo, Baozhu (C-3, C-4)

Hagler, Winston (B-7)
Hamblin, Andrew (C-7)
Hammerschmidt, Ray (F-3)
Haney, Brad (G-1)
Henrick, Clive (F-4)
Herzog, Don (B-4)
Ho, Annie (G-1)
Holbrook, C. Corley (G-1)
Hua, Sui-Sheng T. (A-3, A-4, F-1)

Jain, Ashok K. (D-8)
Ji, Cheng (F-2)
Jurjevic, Zeljko (B-5, B-6)

Kaufmann, Benjamin (C-7)
Keller, Nancy P. (D-6, E-3, F-10)
Kisyombe, Charles T. (A-6)

Lambert, Robert J. (C-5)
Lee, Dewey (B-4)
Lee, Sung-Eun (F-5, F-6)
Lee, Li-Wei (E-6)
Light, Douglas M. (F-4, F-5)
Linz, John E. (E-6, E-7)
Lynch, Robert E. (C-3, C-4)

Mahoney, Noreen (F-6, F-7, F-9)
Mashos, Karol (G-2)
McAlpin, Cesaria E. (A-1, A-3)
McGranahan, Gale (F-9)
Mei, Chuansheng (D-4)
Meredith, Filmore (B-5)
Merrill, Glory (F-4)

Molyneux, Russell (F-7, F-9)
Moore, Kenneth G. (D-3)
Mubatanhema, Wellington(B-5, C-1, G-1)

Naidoo, Gnanambal (C-6, C-7)
Nguyen, Cecilia T. (B-2)
Norton, Robert A. (F-2)

Ozias-Akins, Peggy (D-1, D-2)

Paul, Chandra (C-7)
Payne, Gary (B-7, D-3, E-4, F-1)
Platis, Crystal E. (A-3)

Rajapaska, Dayananda (F-4)
Rajasekaran, K. (D-7)
Ramesh, Marilee (E-5)
Reynolds, Kathy (F-4)
Rocheford, Torbert R. (C-6, C-7)
Roitman, James Roitman (F-4, F-8)
Roze, Ludmila (E-7)
Rozzi, Rebecca D. (C-5)

Severns, Dina E. (C-5, C-7)
Shih, Betty Y. (G-2)
Shimizu, Kiminori (E-3)
Snook, Maurice E. (C-3, C-4)
Stromberg, Kurt D. (D-7)

Trail, Frances (F-3)

Velasquez, Luis (F-3)

Wauchope, Don (G-1)
Weissinger, Arthur K. (D-3)
White, Donald G. (C-5, C-6, C-7, D-4)
Wiatrak, Pawel (B-4)
Wicklow, Donald T. (A-2, A-3, F-2)
Widholm, Jack M. (D-4)
Widstrom, Neil W. (B-5, C-1, C-3, C-4)
Williams, W. Paul (C-2, B-3)
Wilson, David M. (A-6, B-4, B-5, B-6, C-1 G-1)
Wilson, Jeffrey P. (B-6)
Wilson, Richard (D-6)
Windham, Gary L. (B-3, C-2)
Woloshuk, Charles P. (E-1, E-5)
Wright, David L. (B-4)

Xu, Haixin (F-3)

Yang, Hongyu (D-1, D-2)
Yu, Jiujiang (E-1, E-2, E-8)

Zeringue, Jr., Hampden J. (G-2)
Zhang, Zhongjun (C-4)

APPENDIX II

AUTHOR INDEX

Antilla, Larry 83

Baker, James L. 47, 92, 93
Bartelt, Robert J. 29
Basha, S. M. 78
Bayman, P. 53
Beede, Robert 26
Behle, Robert W. 29
Bentley, Walter 26
Berhow, Mark A. 29
Bhatnagar, Deepak 96, 97, 101
Bigelow, Donna M. 87
Boston, Rebecca S. 73
Bourgeois, Robert 65
Boyd, Maria L. 94
Bressan, R. A. 60
Brock, Jason H. 16
Brown, Robert L. 11, 75, 89
Buckley, Paul M. 17, 31
Buntin, David 32
Bush, Brian 35
Butrón, Ana 9, 18
Buttery, Ronald 50
Byrne, P. F. 10

Calvo, Ana 43, 56
Campbell, Bruce C. 22, 39, 50, 51, 52, 55
Cardwell, Kitty 11
Carson, Marty 35
Cary, Jeffrey W, 64, 65, 75, 77, 101
Casper, Howard 34
Chang, Perng-Kuang 97, 101
Chen, Zhi-Yuan 11, 75
Chlan, Caryl A. 65
Cleveland, Thomas E. 9, 11, 61, 64, 65, 66, 75, 77, 89, 96, 101
Coca, M. 60
Cole, Richard J. 85
Cotty, Peter J. 82, 83, 86, 87, 94
Cubeta, Marc 35

Damann, K. E. 75
Damsz, B. 60
Dandekar, Abhaya M. 5, 63
Davidson, Jr., J. I. 106
Davis Frank M. 31
Dorner, Joe W. 85, 88
Doster, Mark 25, 26
Dowd, Michael K. 86
Dowd, Patrick F. 29, 48
Doyle, James 25
Du, Wanglei 47, 98
Duvick, Jonathan P. 29

Ehrlich, Kenneth C. 101
Escobar, Matthew 63
Evans, Bonnie D. 16, 33

Fakhoury, Ahmad 44
Felts, Daniel 25
Flores-Espiritu, Melanie 47, 93
Forbes, Amy M. 7, 21, 22
Franke, Kim K. 13

Gardner, Harold 43, 56, 76
Gill, Ravinder 69, 71, 72
Goldhamer, David 25
Gradziel, T. M. 5, 55
Greene, Ronald L. 88
Grimm, Deborah 105
Guo, Baozhu 9, 10, 18, 19

Haddon, W. F. 27
Hagler, Winston 35
Hamblin, A. 22
Hammerschmidt, Ray 49
Haney, Brad 104
Hassapis, Chris 104
Hasegawa, P.M. 60
Henrick, Clive 50
Herzog, Don 32
Ho, Annie 104
Holbrook, C. Corley 13, 15, 104
Horn, Bruce W. 85, 88
Hua, Sui-Sheng T. 47, 81, 92, 93
Huh, G.-H. 60

Ingram, Keith T. 13, 15

Jacks, Tom J. 64
Jain, Ashok K. 78
Jaynes, J. 66
Ji, C. 48
Jurjevic, Zeljko 33, 34

Kaufmann, B. 22
Keller, Nancy 43, 56, 76
Kendra, David F. 29
Kisyombe, Charles T. 95
Kling, Jennifer 11

Lagrimini, L. Mark 29
Lamb, M. C. 106
Lambert, R. J. 20
Lee, Dewey 32
Lee, Sung-Eun 39, 51, 52
Light, Douglas M. 51
Leslie, Charles 63
Light, Douglas 39, 50
Linz, John E. 100
Liu, Y.-S. 66
Lynch, Robert E. 9, 10, 18, 19, 69

Narasimhan, M. 60

Mahoney, Noreen 39, 52, 53, 55
Manuel, Renee 65
Marshall, L.C. 4
Martinson, C. A. 6
Maskos, Karol 105
Matheron, Michael E. 13
McAlpin, Cesaria E. 90, 92
McGranahan, Gale H. 55, 63
McGuire, Michael R. 29
McMullen, M. D. 10
Mei, Chuansheng 74
Mellon, Jay E. 86
Menkir, Abebe 11
Meredith, Filmore 33
Merrill, Glory 39, 50
Michailides, Themis 25, 26
Mirkov, Eric 66
Molid, Gary 29
Molyneux, Russell J. 39, 53, 55
Moonan, Francis 66
Moore, Kenneth G. 73
Morgan, David 25

Moyne, Anne-Laure 61
Mubatanhema, Wellington 16, 33, 104
Murray, J. 66

Naidoo, Gnanambal 7, 21, 22
Nelson, Merritt R. 87
Norton, R. A. 42, 48

Orum, Thomas V. 87
Ozias-Akins, Peggy 69, 71, 72

Pateña, George F. 15
Paul, Chandra Paul 7, 22
Payne, Gary A. 35, 46, 47, 73, 89, 98
Pearson, Thomas 27
Platis, Crystal E. 92

Salzman, R. 60

Rajapaska, Dayananda 50
Rajasekaran, Kanniah 64, 65, 77
Ramesh, Marilee A. 99
Reynolds, Katherine 50
Rocheford, Torbert R. 7, 21, 22
Roitman, James N. 39, 50, 54
Roze, Ludmila 100
Rozzi, Rebecca D. 7, 20

Scanlon, S. 66
Schatzki, Thomas F. 27
Severns, Dina E. 7, 20, 22
Shih, Betty Y. 105
Snook, Maurice E. 9, 10, 18, 19
Sternitski, D. A. 106
Stromberg, Kurt D. 64, 77

Tebbets, Steven 63
Tonder, Jana 61
Trail, Frances 49
Trucksess, Mary W. 28
Tuzun, Sadik 61

Uratsu, Sandie 63

Vail, Patrick 63
Velasquez, Luis 49
Veronese, P. 60

Wassom, James 74
Wauchope, R. Donald 13, 104
Weissinger, Arthur K. 66, 73
White, Donald G. 7, 11, 20, 21, 22, 29, 74
Wiatrak, Pawel 32
Wicklow, D. T. 4, 48, 91
Widholm, Jack 74
Widstrom, Neil W. 9, 10, 16, 18, 19, 33
Williams, W. Paul 17, 31, 89
Wilson, Jeffrey P. 34
Wilson, David M. 9, 13, 16, 28, 32, 33, 34, 95
Wilson, Richard 76
Windham, Gary L. 17, 31, 89
Woloshuk, Charles P. 44, 96, 99
Wright, David L. 32

Xu, Haixin 49
Xue, Huiqin 13

Yang, Hongyu 69, 71, 72
Yu, Jiujiang Yu 96, 97 101

Zeringue, Jr., Hampden J. 105
Zhang, Z. J. 10, 19

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